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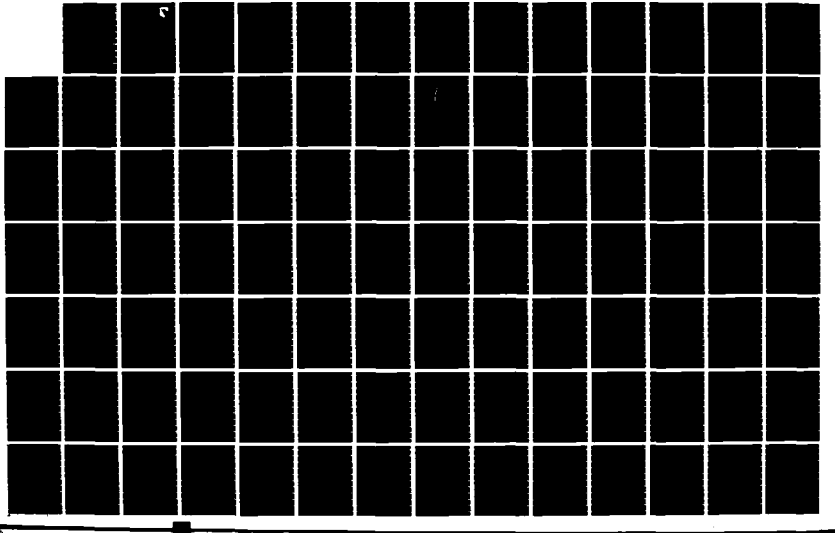

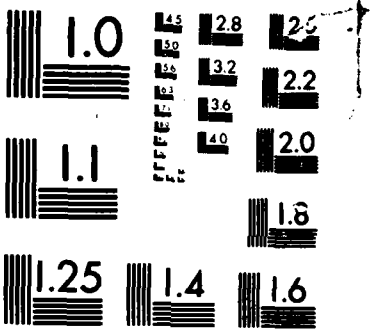
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IN VIVO ASSESSMENT OF MECHANISMS CONTROLLING CORNEAL HYDRATION(U)

MELVIN R. O'NEAL, O.D., Ph.D., MAJOR, USAF

HARRY G. ARMSTRONG AEROSPACE MEDICAL CENTER
RESEARCH LABORATORY

JANUARY 1986

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This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

The voluntary informed consent of the subjects used in this research was obtained as required by Air Force Regulation 169-3.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

CHARLES BATES, JR.
Director, Human Engineering Division
Armstrong Aerospace Medical Research Laboratory

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Both passive and active mechanisms function in control of corneal hydration. These mechanisms have primarily been determined from in vitro animal studies; however, the mechanisms controlling human corneal hydration have yet to be assessed. To investigate these mechanisms, corneal hydration was increased using a contact lens induced hypoxic stress. Following lens removal, the rate of corneal thickness recovery was monitored under various environmental conditions.

Recovery of the cornea to baseline thickness follows a nonlinear time course, with the rate of recovery decreasing as the cornea thins. For initial swelling of 40-54 µm, 55-69 µm and 70 µm and above, the time to reach baseline thickness was 1.5, 2.0, and 2.5 hours, respectively. The active endothelial pump and passive evaporation components of this recovery were studied by inducting edema and monitoring the subsequent decrease in corneal thickness both with and without evaporation during open

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In Vivo Assessment of Mechanisms Controlling Corneal Hydration (U)

19. ABSTRACT

and closed eye conditions, respectively. For open eye recovery from 60 um of swelling, the edothelial pump provides 20%, while the osmotic thinning caused by tear evaporation contributes 80% of recovery. During recovery, the rate of water evaporation from the anterior corneal surface remained relatively steady at 2.5 ul/cm^2 x hr. The measured recovery rates with the eye closed showed good correspondence (within 2 um/hr) to those calculated for the endothelial pump. This suggests that the endothelial pump functions at one speed and that the "pump-leak" theory of corneal hydration control is applicable for the human cornea.

Endothelial function may be affected by the endothlial cell loss and increased variability in cell shape and size (polymegathisms) that accompany aging. Age-related changes in endothelial function were assessed by comparing the corneal recovery and endothlial morphology between a group of younger (mean age, 26.7 yrs) and older (65.7 yrs) subjects who were free of clinically assessible corneal disease. Recovery rates were significantly slower for the older vs younger subjects during both closed and open eye recovery. When each morphological characteristic was isolated, the only significant correlation found was between the coefficient of variation in cell area and the rate of recovery during eye closure, $r = -0.66$ (p 0.05). These data suggest that endothelial pump function decreased approximately 10% by age 65 and indicates a possible link between endothlial morphology and function.

PREFACE

This document was produced as a Doctor of Philosophy Degree dissertation in corneal physiology for the Physiological Optics program of the School of Optometry, University of California - Berkeley while on assignment in the Civilian Institute program of the Air Force Institute of Technology. My advisor for this effort was Professor Kenneth A. Polse of the University of California.

Since the content of this dissertation should be of interest to a wide range of individuals involved in corneal research, the dissertation has been produced as a Harry G. Armstrong Aerospace Medical Research Laboratory technical report. The document does not follow standard technical report format since it was originally a dissertation.

This document includes additional pages in the results and discussion sections of Chapter 5 subsequent to further data analysis following submission of the original dissertation to the university.

ACKNOWLEDGEMENT

It is with great pleasure and utmost respect that I acknowledge the years of direction, support, and encouragement from my advisor, Professor Kenneth A. Polse.

I also wish to thank the members of my committee, Professors Robert B. Mandell, Richard J. Brand, Morton D. Sarver, and Irving Fatt, for their time and counsel.

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A special thank you is reserved for Dr. Donald D. Dunton, Colonel, USAF, who's support enabled me to enter the Air Force sponsored graduate program. My thanks to Captain Robert Perry, program manager, for the assistance he provided me these past few years.

My sincere thanks to the many persons who participated as subjects.

Finally, to my wife, Mary, words cannot express my appreciation for your patience, encouragement, understanding, and support.

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CHAPTER 1

INTRODUCTION

Normal corneal hydration is necessary for the maintenance of optical transparency [Mishima, 1968]. Both passive and active mechanisms function in control of corneal hydration [Maurice, 1962]; particularly those located in the primary control layer, the endothelium, and the evaporation of fluid from the epithelial surface. These mechanisms have primarily been determined from in vitro animal studies. The assessment of the hydration control mechanisms for the in vivo human cornea are needed to better understand and determine the factors affecting the cornea that result in corneal decompensation and loss of transparency.

This thesis represents a series of three investigations designed to study environmental factors and endothelial mechanisms which affect and control hydration of the normal human cornea. To make these investigations it is necessary to first alter corneal hydration in a manner that does not damage the cornea or affect endothelial function. Part I was a study of the use of contact lenses to induce this edema while insuring corneal integrity and subject safety. Following the inducing of corneal edema, the rate of recovery under specific environmental conditions was conducted in Part II to assess the control mechanisms in normal corneas of young subjects. In Part III, a group of older subjects was measured to

evaluate the effect of normal aging on endothelial function. These studies led to the development of an Endothelial Function Test (EFT), in which an increase in corneal hydration is induced by using a hydrogel contact lens to create hypoxia at the epithelial surface and then monitoring the subsequent decrease in corneal thickness during recovery.

Oxygen insufficiency (hypoxia) at the epithelial surface is known to cause corneal swelling and decrease corneal transparency [Smelser, 1952]. The corneal swelling due to hypoxia is presumeably caused by an increase in the production of lactate, which accumulates in the stroma, creating an osmotic imbalance and increased stromal hydration [Klyce, 1981]. It is not known if an increased level of lactate has a toxic effect on corneal function. Epithelial hypoxia has also been reported to decrease the oxygen tension in the aqueous humor [Stefansson et al, 1983], and an effect of hypoxia directly on the endothelium has been suggested [Weissman and Fatt, 1982]. If endothelial compromise occurs in addition to the metabolic by-product, then one might expect a change in the corneal edema response at some level of hypoxia (ie. a biphasic response). Development of the complete hypoxia-swelling response relationship for the normal human cornea would allow an assessment of the possible interference of hypoxia on endothelial function.

Hypoxia can be created at the epithelial surface by using a goggle to pass gas of low oxygen tension across the eye. Studies using the goggle method have found substantial variability in the degree of edema created and have reported a wide range for the minimum oxygen level to prevent corneal edema [Polse and Mandell, 1970; Mandell and Farrell, 1980; Holden et al, 1984]. Differences in gas temperature, hydration, and flow rate could contribute to the disparity in their findings; and reduces the usefulness of this technique in a clinical setting. An alternate method of creating epithelial hypoxia is the use of contact lenses worn while the eye is closed [O'Neal et al, 1983]. This technique was evaluated as a method to obtain a more stable hypoxic condition without affecting corneal integrity.

The individual mechanisms and functions of the corneal layers which affect corneal hydration operate in both an active and passive manner. The endothelium is the site of the ion pump mechanism for active removal of water from the cornea [Maurice, 1972], while both the epithelium and endothelium offer a passive resistance to the flow of water into the cornea [Mishima and Hedbys, 1967] caused by the stromal tendency to imbibe water and swell [Hedbys and Dohlman, 1963]. The passive fluid evaporation from the anterior cornea surface can also have a considerable effect on corneal thickness [Mishima and Maurice, 1961]. These active and passive mechanisms were combined by Maurice [1962] into the "pump-leak" theory of corneal hydration control. However,

these mechanisms have primarily been determined from *in vitro* animal studies, and there have been few *in vivo* studies which have assessed the hydration control mechanisms for the human cornea. Further data is needed to assess the applicability of the basic animal studies to the *in vivo* human cornea.

Endothelial function, and corneal hydration, may be affected by the endothelial cell loss and increased variability in cell shape and size (polymegathism) that accompany normal aging [Laing et al, 1978; Laule et al, 1978]. However, no correlation was found between endothelial cell density and corneal thickness [Bourne and Kaufman, 1976]. In a group of older patients, Rao et al [1984] found a correlation between the presurgical degree of polymegathism and the level of edema following intraocular lens implant surgery. The effect of aging on the endothelial barrier function has been assessed using fluorescein, with Bourne et al, [1984] reporting no correlation between age and the endothelial permeability to fluorescein; while Sawa et al, [1983] found that younger subjects had a higher permeability than older subjects. These studies suggest that the endothelial barrier function does not decrease with aging and that endothelial polymegathism and function are related. This implicates the endothelial pump as the mechanism affected by aging; however, there have been no studies to determine the correlation between endothelial morphology and function.

In summary, in the three studies reported herein, the hydrodynamic responses of the normal human cornea were measured under various environmental conditions. Contact lenses worn during eye closure were used to increase corneal hydration to: (1) determine the dose-response relationship of calculated epithelial hypoxia to corneal swelling, and (2) assess the effect of hypoxia on endothelial function. Corneal hydration recovery following hydrogel lens induced hypoxia was monitored to: (3) evaluate the active and passive mechanisms which control corneal hydration in the in vivo human cornea, (4) assess the effect of hypoxia on the subsequent recovery, (5) determine the relative contribution of the endothelial pump and evaporation components of corneal recovery, (6) assess the applicability of the "pump-leak" theory of corneal hydration control for the in vivo human cornea, (7) assess age-related changes in the endothelial pump mechanism, and (8) determine the normative time course of corneal hydration recovery for younger and older subjects.

The results of these studies indicate that the epithelial hypoxia-swelling response relationship is a smooth, monotonic function, which suggests that inducing corneal hypoxia using a contact lens does not affect endothelial function (Chapter 3). A minimum oxygen requirement of 40 mmHg, determined using the contact lens technique and calculations, is necessary to prevent hypoxic edema. Inducing increased corneal hydration using a hydrogel contact lens to create hypoxic stress did not appear to affect the subsequent hydration recovery

(Chapter 4). For open eye recovery from 60 m of swelling, the endothelial pump provides 20%, while the osmotic thinning caused by tear evaporation contributes 80% of recovery. Using calculations to estimate a predicted rate of recovery due to the endothelial pump, the comparison of measured vs calculated recovery rates during closed eye recovery suggests that the endothelial pump functions at one speed regardless of stromal hydration, and that the "pump-leak" theory of corneal hydration control is applicable for the in vivo human cornea. Comparison of the time course of corneal hydration recovery between an older and younger group of subjects suggests that the endothelial pump rate decreases approximately 10% by age 65 (Chapter 5). Endothelial cell photomicrograph analysis indicates a possible link between endothelial morphology and function.

CHAPTER 2

GENERAL METHODS

2.1 Pachometry

Corneal hydration is linearly related to corneal thickness [Hedbys and Mishima, 1966], therefore by monitoring changes in corneal thickness the fluid movement from the cornea can be determined. It is convenient to note that for every micron of change in corneal thickness per hour, the fluid flux is 0.1 $\mu l/cm^2$ x hr.

Central corneal thickness was measured using a Haag-Streit Pachometer type I, which consists of a main attachment and split image eyepiece placed in the right ocular. The type I device was attached to to a Topcon Biomicroscope and connected to a Diagnostics Concepts Electronic Digital Pachometer model 6090. Modifications were made in the pachometer design that were similar to those described by Holden et al [1982], and are shown in Figure 2.1. The angle between the slit beam and right ocular was increased to 75 degrees, which increases the apparent width of the corneal optic section and improves sensitivity. Between the ocular and beam, two red LEDs were positioned at 35 and 70 degrees from the ocular, and were vertically aligned with the junction of the glass plates in the main attachment.

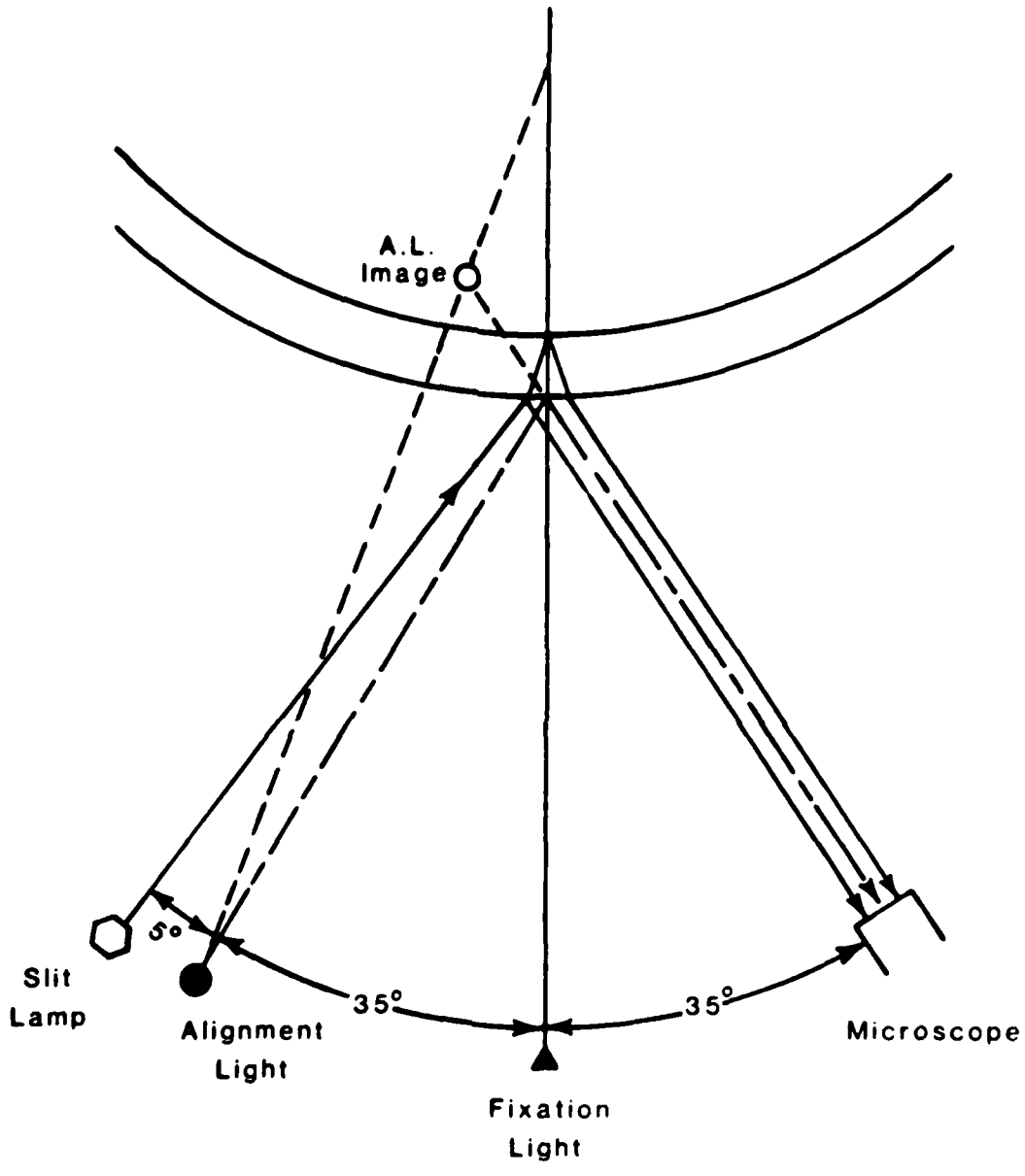

Figure 2.1. Diagram of the optic system for pachometry.

The subject fixated the LED located directly in front, and the LED next to the slit beam was used for alignment purposes. The optic section seen and correct alignment for measurement is shown in Figure 2.2, and is described as follows. Correct alignment is obtained when the alignment LED is seen split equally between the upper and lower images and is located directly in the center of the corneal optic section. In this manner the central position on the cornea where the measurement is taken remains the same for all measurements. Rotation of the upper glass plate in the main attachment displaces the upper half of the optic section in relation to the stationary lower half. The optic section seen includes a bright tear layer followed by a thin optically empty space corresponding to the epithelium. The thick stromal section follows and then the very thin endothelial line at the rear of the section. The measurement was taken when rotation of the glass plate positioned the rear of the optic section in alignment with the rear of the tear image. In this manner the irregularities in the tear surface image and the variation in tear layer thickness were eliminated and the accuracy of the measurement technique improved.

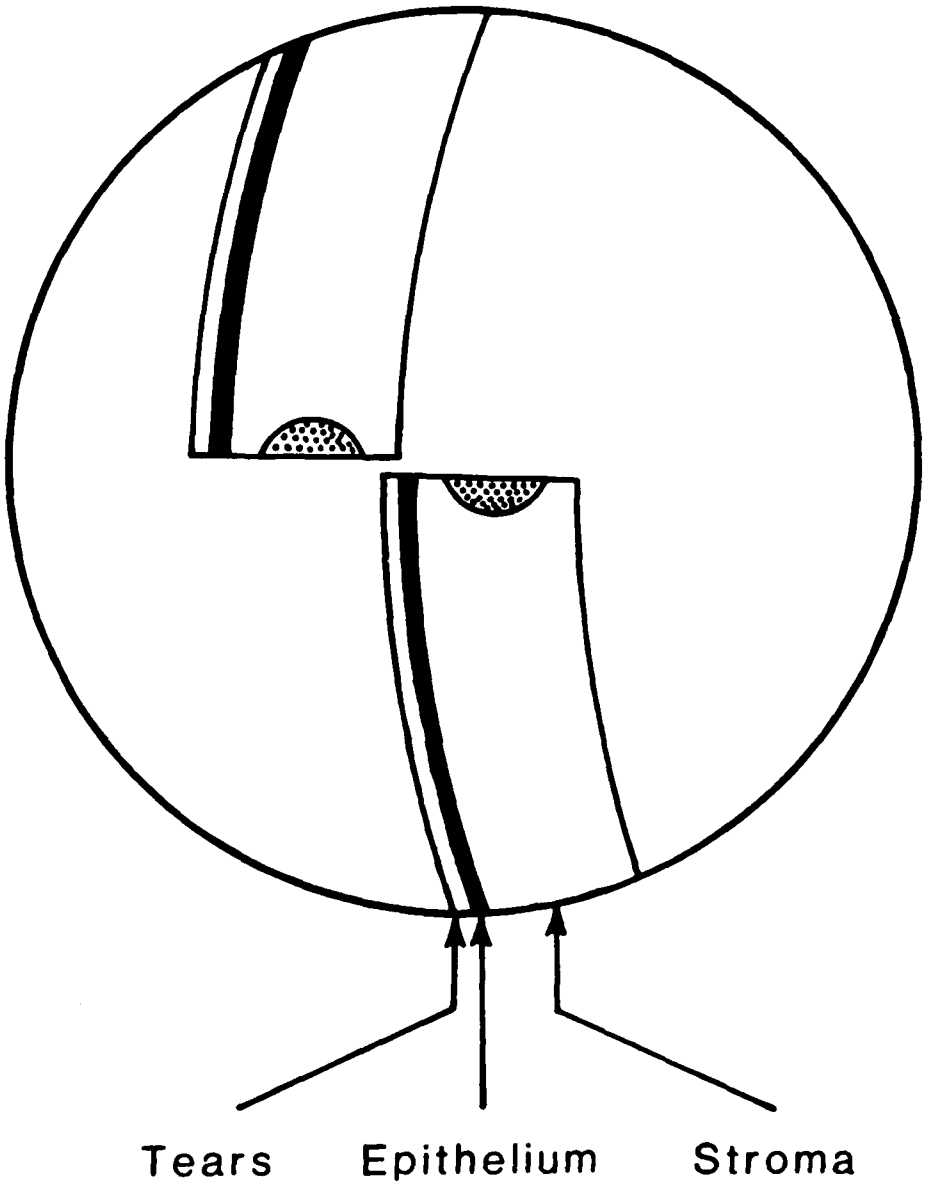

Figure 2.2. Diagram of the optic section in pachometry.

The pachometer was calibrated using PMMA hard contact lenses of known thickness according to the method of Mandell and Polse [1969]. The contact lens is positioned such that the optic section is located at the lens center. The pachometer measurements for the contact lenses were corrected for the differences in refractive indices (n) between a contact lens (n = 1.49) and the cornea (n = 1.376), in which 0.925 x contact lens thickness = corneal thickness.

To insure comparability of all pachometry measurements, a repeatability study was conducted prior to the start of these investigations and at periodic intervals throughout the course of this work. In each repeatability session, three corneal thickness measurements of 10 readings each were made on the same individual. For the ten readings the standard deviation was $\pm$ 4.0 microns, with a usual separation between measurements of 3.0 microns or less. Measurements on the same individual over time were also of the same repeatability. The average corneal thickness of the subjects used in these studies was 509 μm, which is very close to the corneal thickness reported by others [Maurice and Giardini, 1951; Mishima and Hedbys, 1968; Mandell and Polse, 1969].

2.2 Oxygen Transmissibility Measurement

Measurement of the oxygen transmissibility (Dk/L) of the contact lenses used in this study was made using the polarographic procedure originally described by Fatt and St. Helen [1971]. To measure actual contact lenses a curved surface cell was used, which has been described in detail by Fatt [1984] and is shown in Figure 2.3. The gold cathode is surrounded by a hollow silver cylinder anode, into which a thermistor bead is located directly below the surface. The radius of curvature of the cell surface was 7.80 mm, which is close to that of the lenses used in these studies. The polarographic cell is placed in a heated air bath maintained at 35 degrees Centigrade. The polarographic current was amplified by a Schema Versatae Polarographic Amplifier connected to a chart recorder, which indicates when a steady state diffusion of oxygen through the lens has been established.

The hydrogel lenses contain saline solution and can be measured directly on the polarographic cell. However, when rigid lenses are measured a saline saturated membrane must be placed between the lens and cell surface. Cigarette paper having a thickness of 17 μm and Dk/L value of 45×10^{-9} (cm/sec)(ml O_2/ml x mmHg) was used in this study. When the cigarette paper is used, it is a resistance in series with the contact lens and must be subtracted from the reading (L/Dk total - L/Dk paper = L/Dk lens).

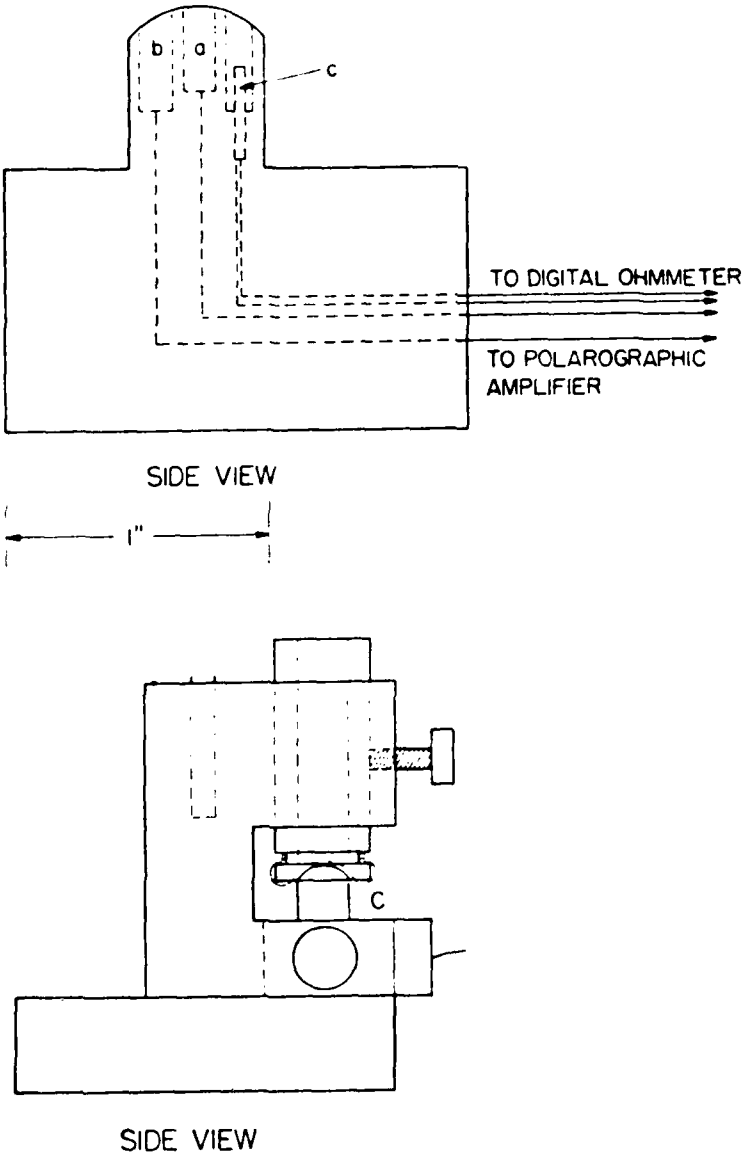

Figure 2.3. Drawing of curved surface polarographic cell. "a" is the gold cathode; "b" is the silver anode in form of cylinder; "c" is a thermistor sealed into the anode. Drawing of the cell holder with polarographic cell "C" in place.

There is also a "dark current" which must be subtracted from the observed current during measurements. This current occurs even when there is no oxygen present, and was measured using a sandwich of two PMMA lens with aluminum foil between the lenses. The dark current was found to be 0.08 microamperes.

The equations used in the derivation of the formula used in the polarographic cell technique include Faraday's Law: $i = nFJ$, and Henry's and Fick's Laws combined: $J = DkAdP/L$. Combining and rearranging gives: $Dk/L = i/nFAdP$; where i is current in microamperes, $n = 4$ (mole e^-/mole O_2), $F = 96{,}500$ (coul/mole e^-), A = surface area of cathode (cm^2), and $dP = 155$ mmHg. Also needed are: 1 coul = 1 amp-sec, 1 amp-sec = 10 microamps, and 1 mole O_2 = 22.4×10^3 ml O_2. Calculation shows that $Dk/L = i \times (1/A) \times 3.74394 \times 10^{-10}$. For the cathode used, the area was calculated from the chord diameter (4.08 mm) and radius of curvature (7.80 mm) using the formula for the area of a segment of a sphere. The cell constant was calculated to be 2.80×10^{-9} (cm/sec)(ml O_2/ml x mmHg) x i = Dk/L.

A repeatability study of the polarographic procedure was conducted by this author and has been published [O'Neal et al, 1983]. In general, this procedure shows good repeatability; when lenses of low oxygen transmissibility (Dk/L) are measured a 2% range is usual and for high Dk/L lenses the range of measurements is approximately 5%.

CHAPTER 3

CORNEAL RESPONSE TO HYPOXIA INDUCED WITH RIGID AND HYDROGEL LENSES WORN DURING EYE CLOSURE

3.1 Summary

Corneal changes were monitored in 14 subjects following 3 hours of eye closure while wearing selected oxygen permeable rigid and hydrogel lenses. The mean increase in corneal thickness ranged from 82.5 to 29.5 μm for rigid lenses with oxygen transmissibilities (Dk/L) between 0.2×10^{-9} and 57.0×10^{-9} (cm/sec)(ml O_2/ml x mmHg), respectively; and ranged from 82.5 to 23.5 μm for hydrogel lenses with Dk/L between 2.5×10^{-9} and 70.0×10^{-9} (cm/sec)(ml O_2/ml x mmHg), respectively. No differences in the amount of swelling between rigid and hydrogel lenses of the same oxygen transmissibility were observed (t test, $p > 0.20$). The smooth, monotonic nature of the hypoxia-corneal swelling relationship suggests endothelial function was not affected. Combining the swelling data for both types of lenses shows that a minimum lens oxygen transmissibility of approximately 75×10^{-9} (cm/sec) (ml O_2/ml x mmHg) is necessary during eye closure to prevent contact lens induced edema. The estimated oxygen tension under a lens with this Dk/L value is 40 mmHg. Recovery of the cornea to baseline thickness follows a nonlinear time course, with the rate of recovery decreasing as

the cornea thins. For initial swelling of 40-54 μm, 55-69 μm, and 70 μm and above, the time to reach baseline thickness was 1.5, 2.0, and 2.5 hours, respectively. Effects on vision, corneal curvature, distortion, and epithelial integrity were not clinically significant during this short period of eye closure.

3.2 Introduction

Normal corneal hydration is necessary for the maintenance of optical transparency [Mishima, 1968]. An adequate supply of oxygen is needed for proper corneal metabolism [Graymore, 1970], and oxygen insufficiency (hypoxia) at the epithelial surface results in corneal edema and loss of transparency [Smelser,1952].

The corneal swelling due to hypoxia is presumeably caused by an increase in the production of lactate, which accumulates in the stroma, creating an osmotic imbalance and increased stromal hydration [Klyce, 1981]. It is not known if an increased level of lactate has a toxic effect on corneal function. Epithelial hypoxia has also been reported to decrease the oxygen tension in the aqueous humor [Stefansson et al, 1983], and an effect of hypoxia directly on the endothelium has been suggested [Weissman and Fatt, 1982]. If endothelial compromise occurs in addition to the metabolic by-product, then one might expect a change in the corneal edema response at some level of hypoxia. Development of the complete hypoxia-swelling response relationship for the normal human cornea would allow an assessment of the possible interference of hypoxia on endothelial function.

Hypoxia can be created at the epithelial surface by using a goggle to pass gas of low oxygen tension across the eye. Studies using the goggle method have found substantial variability in the degree of edema created and have reported a

wide range for the minimum oxygen level to prevent corneal edema, including 11-19 mmHg by Polse and Mandell [1970], 23-37 mmHg by Mandell and Farrell [1980], and 76 mmHg by Holden et al [1984]. Differences in gas temperature, hydration, and flow rate could contribute to the disparity in their findings; and reduces the usefulness of this technique in a clinical setting. It would seem appropriate to investigate other methods of creating epithelial hypoxia to more stabilize the hypoxic environment.

An alternate method of creating epithelial hypoxia is the use of contact lenses. When a contact lens is placed on the eye the oxygen tension at the tear-lens interface is reduced [Efron and Carney, 1981]. During open eye wear, tear exchange and oxygen diffusion through the material of most contact lenses allows sufficient oxygen to reach the cornea to maintain normal corneal metabolism [Tsuda et al, 1981]. However, during eye closure the driving force of oxygen at the anterior lens surface is reduced from 155 mmHg in air to 55-57 mmHg [5] and tear exchange is reduced to a minimum. Contact lenses worn while the eyes are closed would create a stable hypoxic environment that may be useful clinically, and gives an alternative method for determining the minimum oxygen level needed by the cornea.

The amount of oxygen under the lens at the epithelial surface can be calculated using an equation derived by Fatt and St Helen [1971]. The use of lenses having a variety of oxygen transmissibility (Dk/L) values would give a wide range

of oxygen levels under the lens and allow investigation of the complete hypoxia-swelling response relationship. Providing sufficient oxygen to maintain normal metabolism during eye closure requires the contact lens to have a substantially greater Dk/L value than available with most hydrogel materials [Fatt and Chaston,1982]. Alternatively, it is possible to make rigid lenses of silicon/acrylate polymers that have Dk/L values higher than that of present extended wear hydrogel lenses. Combining both hydrogel and rigid oxygen permeable lenses would give a sufficient variety of oxygen transmissibilities to investigate this relationship.

If contact lenses are to be used in a closed eye technique, it is important to also evaluate other factors that may affect the edema response; including whether lens rigidity, movement, and pressure (ie. mechanical interference) alter the corneal swelling response. Also, the effect lenses on the corneal epithelium and corneal curvature during eye closure, and any disturbance in visual acuity must be determined. To make these assessments, lenses made of a soft material (ie. hydrogel lenses) and rigid lenses can be worn under the same conditions, with the corneal responses compared for the two lens types.

Little is known about the time course of recovery after lens removal from corneal swelling. Harris et al [1981] measured recovery on one subject, finding it took 80 minutes and 2 hours to recovery from 8 and 4% swelling, respectively. Tomlinson et al [1981] show recovery data for one subject in

which recovery from 6 and 4% swelling took 3 and 2 hours, respectively. Recently, Johnson et al [1983] presented individual recovery curves for 4 subjects, in which a bi-phasic time course of recovery occured. They reported recovery times of 10 minutes from 2.0% edema and 20 minutes from 4% edema; however, from 14.5% swelling recovery to the 3.5% level took only 30 minutes, then another 50 minutes to reach baseline. There is a wide variation in these reported recovery times. The monitoring of corneal recovery after lens removal from a wide range of edema levels on an adequate number of subjects is needed to allow a more detailed assessment of recovery and to establish the normative time course of corneal hydration recovery.

In this study, the closed eye corneal response was assessed by measuring the change in corneal thickness, corneal curvature, and epithelial integrity following 3 hours of eye closure while wearing selected hydrogel and oxygen permeable rigid lenses. Based on these data, the entire range of the dose-response relationship of calculated epithelial hypoxia to corneal swelling is determined and the possible effect of hypoxia on endothelial function is assessed. The lens oxygen transmissibility necessary to prevent corneal hypoxia during sleep is determined; and using this Dk/L value, the oxygen tension at the tear-lens interface necessary for normal corneal metabolism during contact lens wear is estimated. Corneal hydration recovery was monitored after lens removal to determine the normative time course of recovery from a wide range of initial swelling levels.

3.3 Materials and Methods

Subjects

Fourteen subjects, (six women, eight men; mean age 27.5 $\pm$ 5.5 years, range 20 to 41 years) who were free of ocular disease and had no prior contact lens experience (unadapted) participated in the study. Informed consent was obtained from each subject. A summary of relevant ocular parameters is listed in Table 3.1.

Lenses

Seven rigid (1 PMMA, 6 GPH) and five hydrogel lenses were used. All rigid lenses were 9.2 mm in diameter with a power of -3.00 D. The hydrogel lenses varied in diameter and power. The average thickness of each lens was computed from measurements made across the entire lens for the rigid lenses and across the central 11.0 mm for the hydrogel lenses. The oxygen transmissibility of each lens was measured by the polarographic oxygen sensor method [Fatt and St Helen, 1971], using a curved surface (7.8 mm radius) electrode. Temperature control and lens handling were strictly monitored to increase the repeatability of the measurement [O'Neal et al, 1983]. The physical characteristics of these test lenses are listed in Table 3.2.

Table 3.1. Summary of selected ocular parameters for the 14 subjects.

	Mean	SD	Range
Age (years)	27.5	5.5	20 to 41
Corneal thickness (μm)	509	37	465 to 570
Sphere ref. error (D)	-2.63	1.83	+0.25 to -6.25
Cylinder ref. error (D)	-0.68	0.66	0 to -2.75
Horiz. Keratometry (D)	43.40	1.85	39.87 to 46.62
Corneal toricity (D)	0.46	0.98	2.00 against to 2.00 with

Table 3.2. Physical characteristics of the test lenses.

Number	Name	Material	Dk* 35 °C	Average Thick (mm)	Dk/L+ 35 °C
1	PMMA	PMMA	0.2	0.12	0.2
2	B&L Soflens	38.6++,HEMA	9.8	0.39	2.5
3	Boston Lens III	Sil/Acry	19.1	0.32	6.0
4	B&L Soflens	38.6,HEMA	9.8	0.09	10.5
5	Boston Lens III	Sil/Acry	19.1	0.16	12.0
6	Boston Lens III	Sil/Acry	19.1	0.09	22.0
7	Boston Lens III	Sil/Acry	19.1	0.07	26.0
8	Hydrocurve II	55.0,HEMA	19.2	0.07	26.0
9	Boston Lens IV	Sil/Acry	26.3	0.07	38.0
10	Experimental	70.0,HEMA	38.0	0.08	50.0
11	Paraperm EW	Sil/Acry	57.0	0.10	57.0
12	Experimental	70.0,HEMA	38.0	0.06	70.0

* Oxygen Permeability: $(\times 10^{-11})(cm^2/sec)(ml\ O_2/ml \times mmHg)$

+ Oxygen Transmissibility: $(\times 10^{-9})(cm/sec)(ml\ O_2/ml \times mmHg)$

++ Percent water content

Procedure

Changes in corneal hydration were monitored by measuring central corneal thickness [Hedbys and Mishima, 1966] using a Haag-Streit pachometer adapted to a Topcon biomicroscope and connected to a Diagnostic Concepts electronic digital pachometer unit. The pachometer design was similar to that described by Holden et al [1982]. Each measurement included 15 to 20 readings with a standard deviation of $\pm$ 4.0 microns.

Visual acuity was assessed using a projected Snellen chart. Acuity is expressed as log(MAR), which is the log of the reciprocal of the Snellen fraction. Each change of 0.1 units on the log(MAR) scale is equal to a change of approximately 1 Snellen acuity line. Corneal curvature and mire distortion was measured using a Bausch & Lomb Keratometer. Slit lamp evaluation of corneal edema and epithelial staining was made using a Topcon SL-5D Biomicroscope.

Baseline measurements made prior to lens insertion, included slit lamp examination, corrected visual acuity, keratometry, and pachometry. Following lens insertion, both eyes were closed for 3 hours to obtain steady state corneal swelling [Harris et al, 1981]. Also, no lens was worn during one session to determine the normal physiological closed eye edema. Test lenses were worn on the right eye, with the left eye serving as a control. All subjects were not available to wear each lens; 8 subjects wore lenses 3-8, while 6 subjects wore lenses 1,2, and 9-12. Each subject showed a normal

swelling response to a thick hydrogel test lens worn for 3 hours with the eyes open, indicating the compatability of responses even though all subjects did not wear all lenses. At the end of 3 hours the lens was removed and central corneal thickness readings of both eyes were taken.

Following pachometry, slit lamp examination was repeated to evaluate for corneal edema, striae, and staining. Each observation was graded on a 0-3 scale, corresponding to none, mild, moderate, and severe change, respectively. Spectacle acuity and keratometry measurements were then taken. All measurements were completed within 5 minutes of lens removal.

Recovery of the cornea to baseline thickness was monitored during some sessions by measuring the central corneal thickness every 15 minutes for the first hour and each half hour for the next 3 hours.

3.4 Results

The mean change in central corneal thickness following 3 hours of eye closure while wearing the oxygen permeable rigid contact lenses is shown in Figure 3.1. There is an inverse relationship between the oxygen transmissibility of the lens and the degree of corneal swelling, with the difference between lenses being significant (F test, $p < 0.001$). The mean increase in corneal thickness ranged from 82.5 ± 6.3 µm to 29.5 ± 3.9 µm (16.5 to 5.9%) for lenses with oxygen transmissibilities (Dk/L) between 0.2×10^{-9} and 57.0×10^{-9} (cm/sec)(ml O_2/ml x mmHg), respectively. The curve was fitted by polynomial equation (6th order, $n = 48$, $r = 0.810$). The mean corneal swelling during the control sessions (no lens) was 21.0 ± 4.6 µm (4.2%), range 14.6 to 31.5 µm, and is indicated by the dotted line. (See Appendixes 1 and 2 for the individual swelling data).

Changes in central corneal thickness following lens removal (recovery) are shown in Figure 3.2. The average time to return to baseline corneal thickness was related to the amount of induced edema. The mean recovery time was approximately 1.5, 2.0, and 2.5 hours from initial swelling levels of 40-54 µm, 55-69 µm, and 70 µm and above, respectively. Analysis of recovery rates for different amounts of edema shows that the rate of recovery is the same for any given level of hydration regardless of the initial amount of induced swelling.

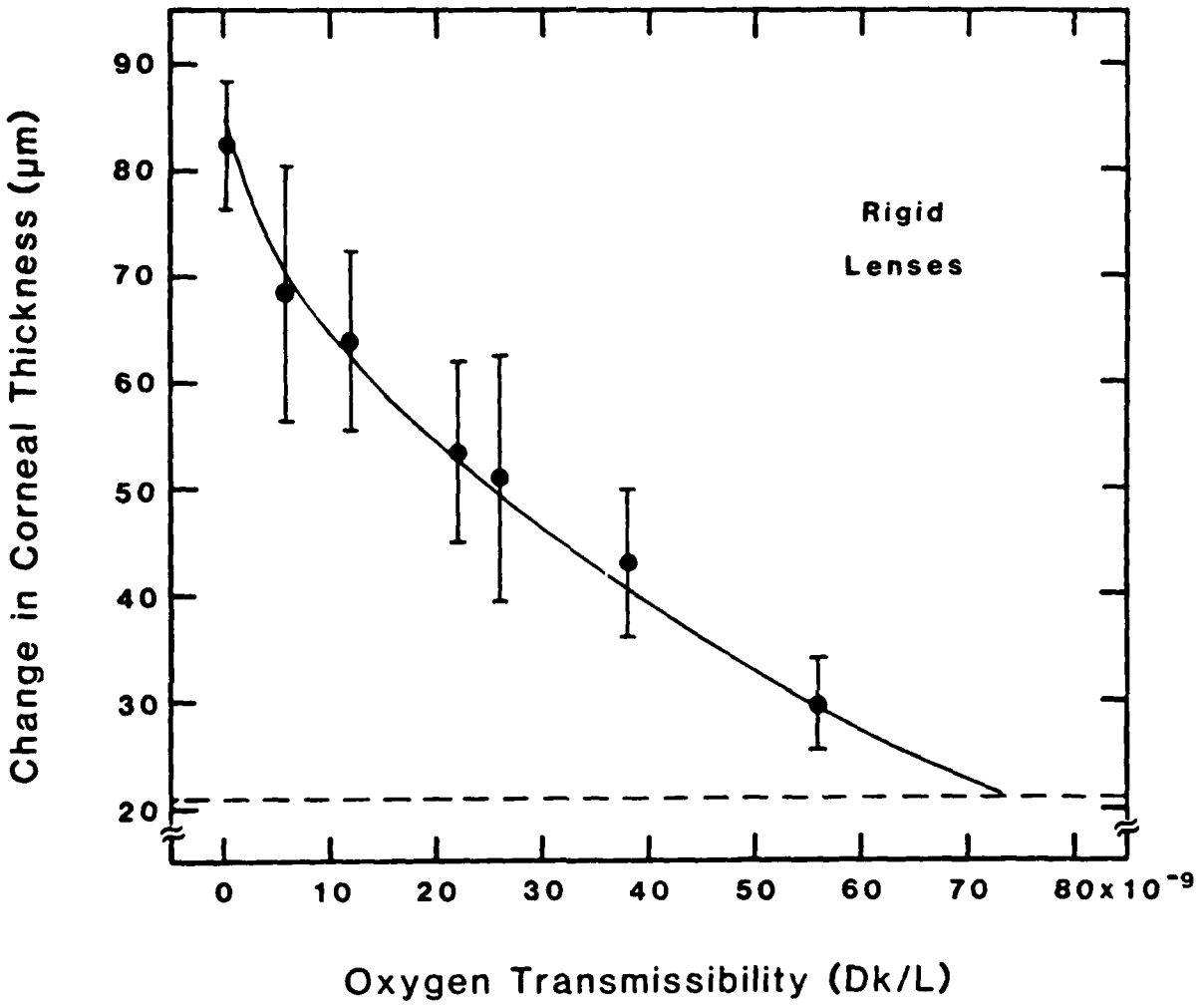

Figure 3.1. Mean change in central corneal thickness vs lens oxygen transmissibility (Dk/L) following 3 hours of eye closure while wearing oxygen permeable rigid contact lenses. Error bars equal $\pm$ 1 SD and line was fitted by polynomial equation. Dk/L units: (cm/sec)(ml O_2/ml x mmHg)

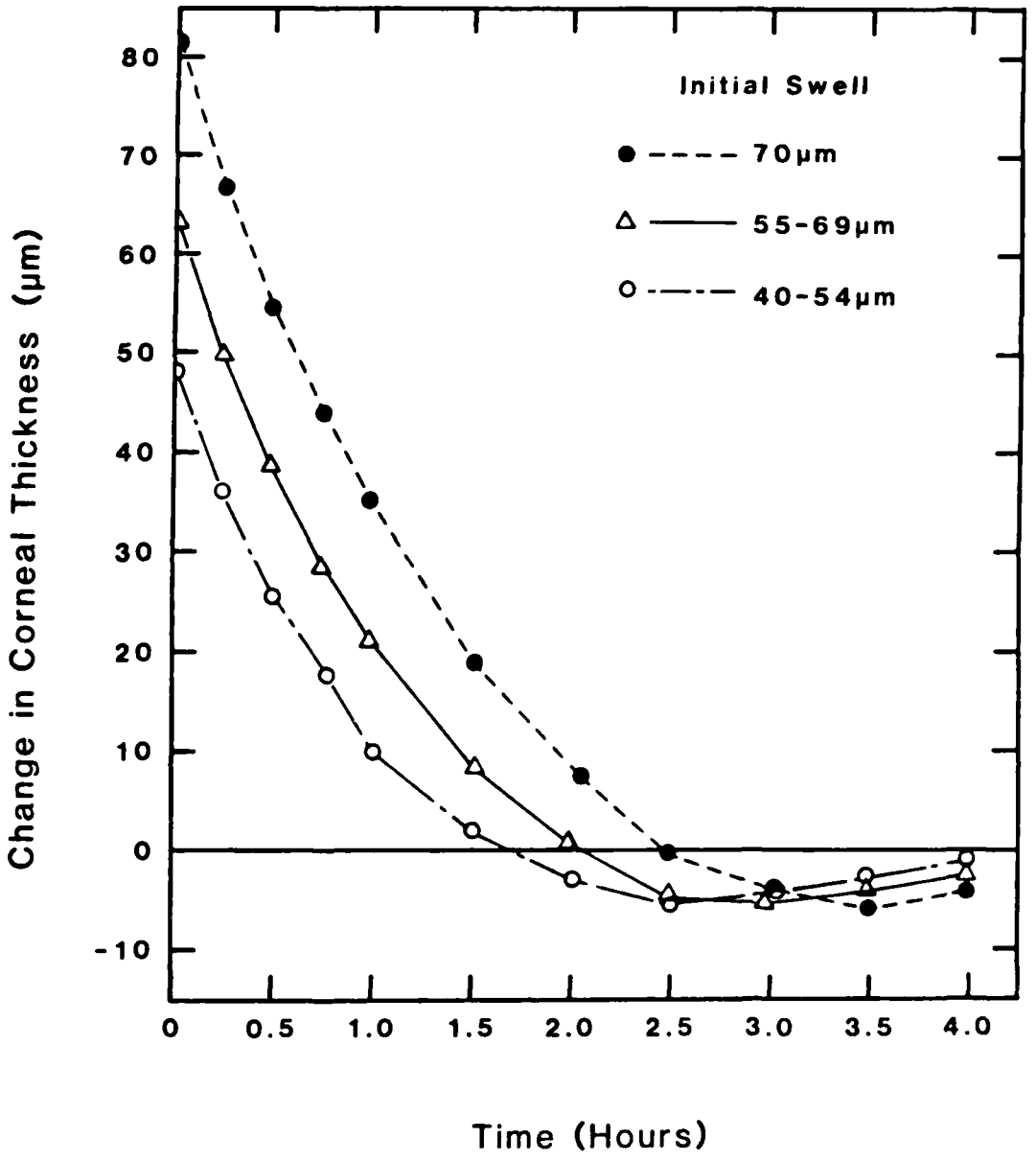

Figure 3.2. Mean change in central corneal thickness vs time (recovery) following lens removal. Initial level of edema was grouped into three ranges of 40-54 µm, 55-69 µm, and 70 µm and above.

For example, in Figure 3.2, the rate of recovery at 30 μm of edema is approximately 30 μm/hour and is the same for all three recovery curves. (See Appendixes 3 - 5 for the individual recovery data).

The mean change in central corneal thickness following 3 hours of eye closure while wearing the hydrogel lenses is shown in Figure 3.3. The mean increase in corneal thickness ranged from 82.5 $\pm$ 6.8 μm to 23.5 $\pm$ 5.1 μm (16.5 to 4.7%) for the lenses with Dk/L between 2.5×10^{-9} and 70.0×10^{-9} (cm/sec)(ml O_2/ml x mmHg), respectively. There was a significant difference in the corneal swelling accompanying these lenses (F test, $p < 0.001$). The curve was fitted by polynomial equation (6th order, $n = 32$, $r = 0.958$).

The swelling response data points for the hydrogel and rigid lenses were fitted by the method of least squares. Comparison of the regression lines shows no difference in the degree of swelling between rigid and hydrogel lenses of the same oxygen transmissibility (t test, $p > 0.20$). The corneal swelling responses for the two types of lenses was, therefore, combined and is shown in Figure 3.4. The curve was fitted by polynomial equation (6th order, $n = 80$, $r = 0.914$), and can be used to predict the effect of lens oxygen transmissibility on corneal hydration during eye closure. The derived polynomial indicates that a minimum lens oxygen transmissibility of approximately 75×10^{-9} (cm/sec)(ml O_2/ml x mmHg) is necessary during closed eye contact lens wear to prevent more corneal swelling than the normal physiological edema (4.2%) that occurs during eye closure.

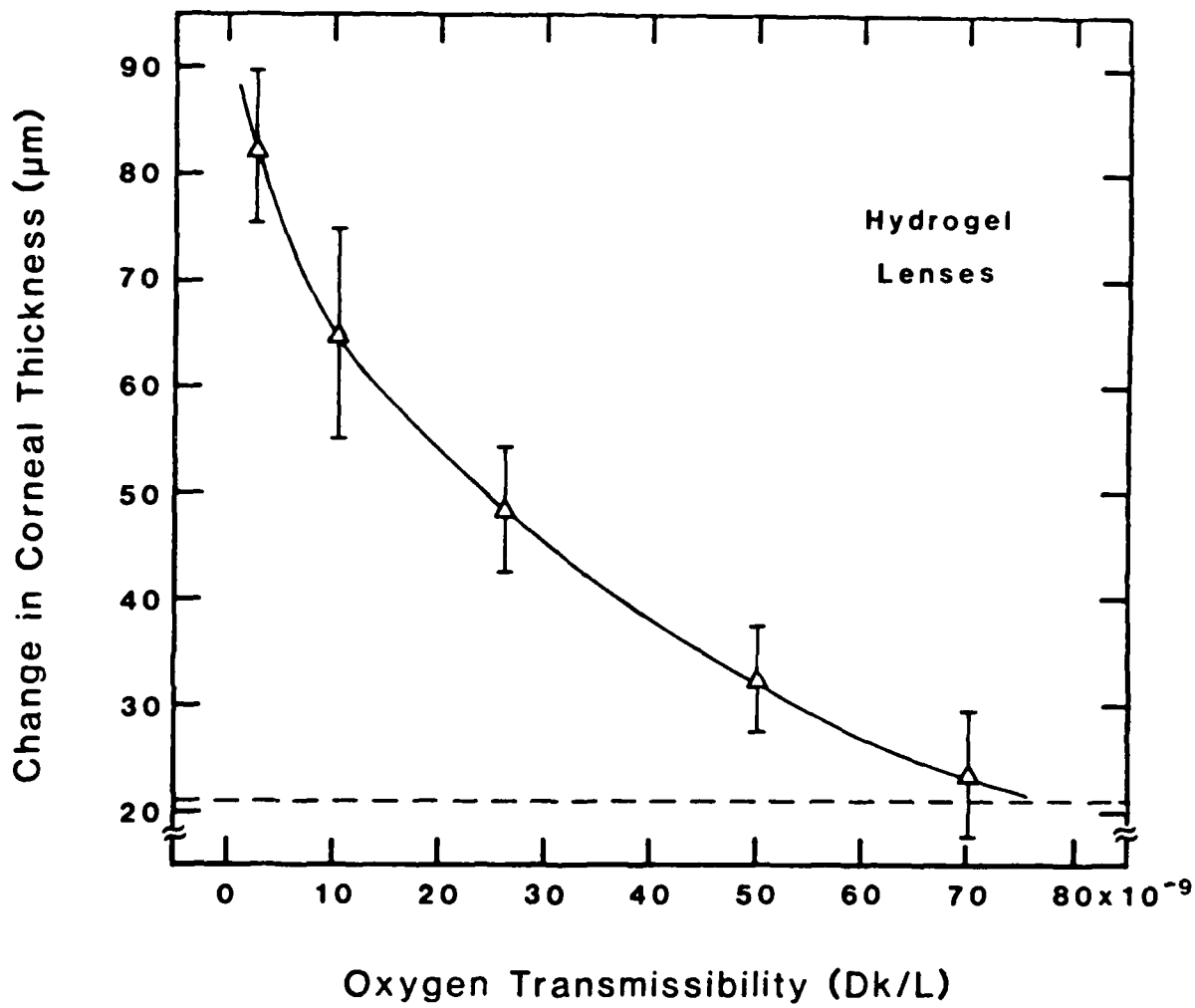

Figure 3.3. Mean change in central corneal thickness vs lens oxygen transmissibility (Dk/L) following 3 hours of eye closure while wearing hydrogel contact lenses. Error bars equal ± 1 SD and line was fitted by polynomial equation. Dk/L units: (cm/sec)(ml O_2/ml x mmHg).

To estimate the oxygen tension under each contact lens for the closed eye condition, calculations were made using a theoretical model proposed by Fatt and St. Helen [1971]:

$$\alpha P^{\frac{1}{2}} = (Dk/L)_{cl} \times (P_a - P),$$

where α is 0.24×10^{-6} ml $O_2/cm^2 \times sec \times (mmHg)^{\frac{1}{2}}$, P_a is the palpebral oxygen tension (55-57 mmHg), $(Dk/L)_{cl}$ is the oxygen transmissibility of the contact lens, and P is the oxygen tension under the lens. This model assumes no tear exchange occurs under the lens when the eye is closed. The estimated oxygen tension under contact lenses for several Dk/L values is shown on the right ordinate of Figure 3.4. These calculations indicate that an estimated oxygen tension of 40 mmHg or greater is necessary for normal corneal metabolism.

The mean corneal and visual changes occuring after 3 hours of closed eye with and without wearing oxygen permeable rigid contact lenses are listed in Table 3.3. In general, corneal curvature changes and corneal distortion were minimal, with only 8 cases of grade 1, 1 case of grade 2, and 1 case of grade 3 distortion ocurring. The corneal curvature changes were variable and do not appear to be related to the amount of swelling. Corneal staining occured in 11 (7 grade 1, 4 grade 2) of the 44 GPH lens sessions, and was typically associated with cases of decentered lenses. Visual acuity was 20/20 in most cases and by the end of the recovery period acuity had returned to 20/20 for all subjects. These changes were similar to those found with the hydrogel lenses.

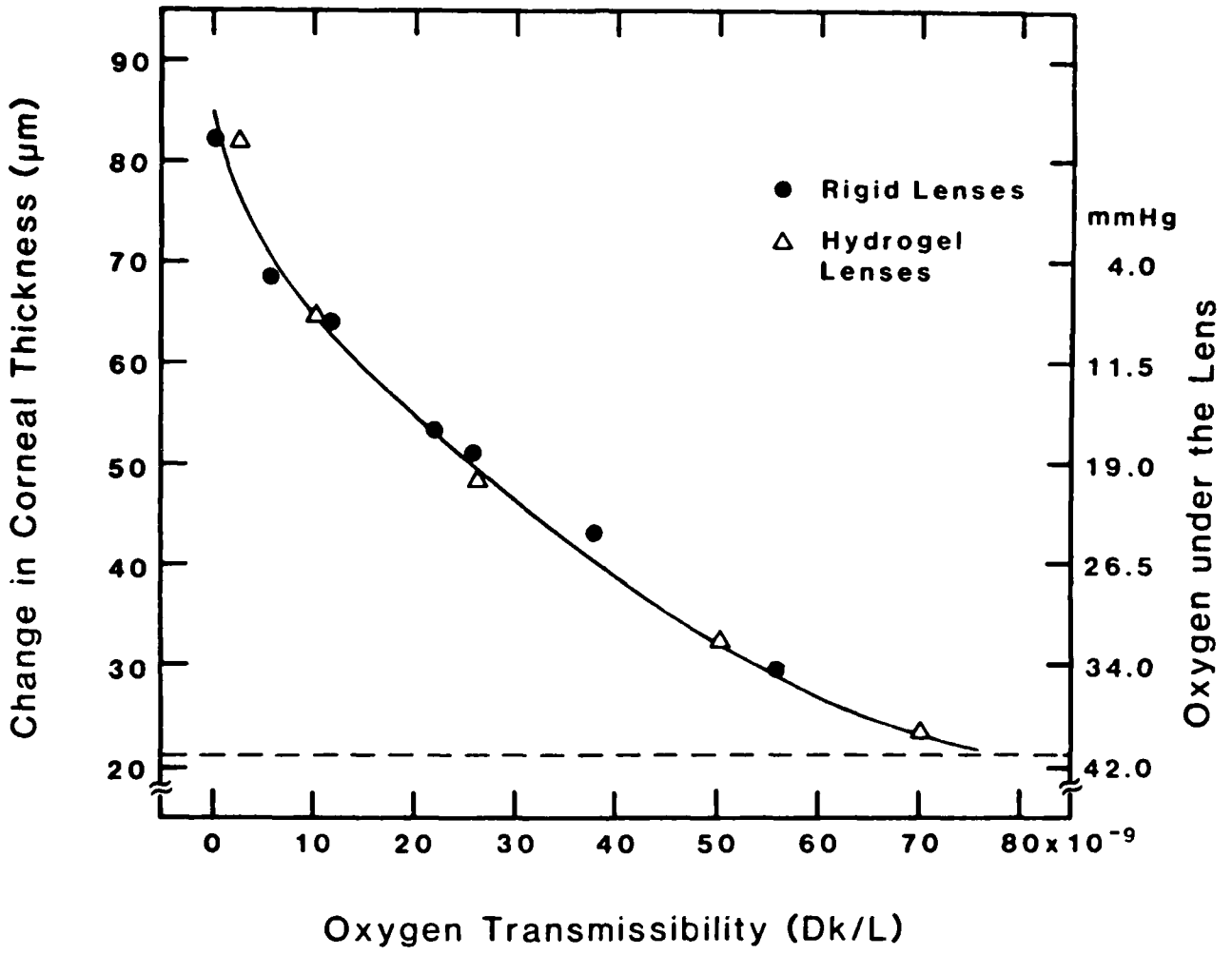

Figure 3.4. Mean change in central corneal thickness vs lens oxygen transmissibility (Dk/L) and oxygen under the lens (mmHg) following 3 hours of eye closure while wearing oxygen permeable rigid or hydrogel contact lenses. Line was fitted by polynomial equation. Dk/L units: (cm/sec)(ml O_2/ml x mmHg).

Table 3.3. Mean visual and physiological response following 3 hours of closed eye with and without wearing PMMA and oxygen permeable rigid contact lenses.

Oxygen transmissibility* Dk/t at 35°C	Corneal curvature (D)		Visual acuity (log MAR)	Corneal edema (grade)	Corneal striae (grade)
	Horizontal	Vertical			
0.2	-0.30 ± 0.36	+0.42 ± 0.23	0.14 ± 0.05	2.50	3.00
6.0	+0.22 ± 0.38	+0.39 ± 0.45	0.07 ± 0.06	1.13	2.13
12.0	-0.19 ± 0.38	+0.19 ± 0.37	0.06 ± 0.07	0.63	1.75
22.0	-0.02 ± 0.20	+0.23 ± 0.42	0.01 ± 0.03	0.25	1.13
26.0	-0.05 ± 0.21	+0.17 ± 0.33	0.04 ± 0.05	0	0.88
38.0	-0.19 ± 0.17	0 ± 0.25	0 ± 0	0	0.57
57.0	+0.15 ± 0.20	+0.07 ± 0.27	0 ± 0	0	0.16
Control	-0.01 ± 0.14	+0.05 ± 0.11	0 ± 0	0	0

* $\times 10^{-9}$ (cm/sec) (ml O_2/ml × mmHg)

Corneal edema, as assessed with the slit lamp, was visible in only a few of the rigid lens sessions, appearing as a diffuse, nonlocalized haze. Corneal striae occured either as single lines, or more commonly in groups of lines. Corneal striae was measured as grade 1 for 1-6 lines; grade 2 for 7-11 lines, and grade 3 for 12 lines or greater. For the rigid lens sessions, the grade of corneal striae correlated well with the amount of corneal edema ($r = 0.83$). Corneal swelling of <40 µm (8%), 40-50 µm (8-10%), 55-65 µm (11-13%), and 70 µm (14%) and above was usually accompanied by grades 0, 1, 2, and 3 corneal striae, respectively.

3.5 Discussion

The amount of corneal swelling following 3 hours of closed eye lens wear decreased as lens oxygen transmissibility (Dk/L) increased. These data indicates a *minimum* lens Dk/L of approximately 75 x 10^{-9} (cm/sec)(ml O_2/ml x mmHg) is necessary to prevent the corneal swelling that results from contact lens wear during eye closure.

Corneal swelling induced by hypoxia tends to reach steady state in 3 hours [Harris et al, 1981] and therefore a 3 hour test period provides a reasonable indicator of overnight edema accompanying extended wear. However, 3 hours of eye closure under laboratory conditions is an approximation of the normal overnight, sleep condition. The rapid eye movements (REM) and occasional eye openings that occur during normal sleep may allow some tear exchange (eg. increased oxygen) beneath the oxygen permeable rigid lens, which may reduce the amount of swelling. Also, these findings are based on a relatively small sample size, and testing with more subjects and longer closed eye duration are needed to verify these results. These swelling responses, however, are in agreement with those reported for 6 hours of eye closure [Sweeney and Holden, 1983] and following sleep during extended-wear [Holden et al, 1983].

An equation based on average corneal properties was used to estimate the oxygen tension at the tear-lens interface for closed eye wear of contact lenses having different oxygen transmissibilities. If the decreased oxygen level or

metabolic by-product had interfered with endothelial pump function then a sudden change in the swelling response (ie. biphasic) would be expected. However, the epithelial hypoxia-corneal swelling response followed a smooth, monotonic relationship, suggesting that a steady increase in a metabolic by-product, such as lactate, induced the edema osmotically rather than by a direct effect on the endothelial pump mechanism. The results indicate that an oxygen tension of 40 mmHg is necessary to prevent hypoxic edema. This oxygen level is in agreement with open eye measurements showing no edema for wear of a soft lens having a Dk/L of 22×10^{-9} (cm/sec)(ml O_2/ml x mmHg) [Sarver et al, 1981] and corresponding 5.0% equivalent oxygen percent (EOP) under the lens [Fatt and Chaston, 1982].

The minimum oxygen requirement, 40 mmHg, determined using the contact lens technique is higher than the thresholds determined by Polse and Mandell [1970], 11 - 19 mmHg, and by Mandell and Farrell [1980], 23 - 37 mmHg, who used a goggle to induce hypoxia. These differences may be due to increased metabolic activity accompanying contact lens wear, higher corneal temperature when a contact lens is worn with the eye closed, or differences between inducing corneal hypoxia with a goggle as compared to contact lenses. During the goggle experiments the flow of gas across the cornea may result in an increased effect of evaporation on corneal thickness and a limited swelling response. On the other hand, this oxygen threshold is lower than the 76 mmHg determined by Holden et al

[1984]. The difference may be due to variation in hydration of the gases in the goggle causing fluctuations in swelling response that add to the effect of the oxygen tension.

Approximately the same amount of corneal swelling resulted when either oxygen permeable rigid or hydrogel lenses with similar oxygen transmissibility were worn during 3 hours of eye closure. This similarity of swelling responses between rigid and soft lenses of the same Dk/L suggests that the swelling is caused by corneal hypoxia rather than mechanical interference and that there is little or no tear exchange under either lens type during eye closure. The changes in vision, corneal curvature, distortion, and epithelial integrity were not clinically significant for wear of the rigid lenses during this short period of eye closure, and were similar to that commonly reported for closed eye wear of hydrogel lenses. Corneal molding and epithelial staining may increase with long term closed eye wear and needs additional study.

The ability to assess the corneal swelling accompanying closed eye wear of rigid lenses without the use of a pachometer is of clinical importance. The central corneal edema observed in daily wear of rigid lenses [Mandell, 1981] was not usually seen with closed eye wear and therefore is not a useful indicator of edema. However, corneal striae, which is observed in open eye wear of hydrogel lenses [Polse et al, 1975] but not rigid lenses, did occur with rigid lenses during the closed eye condition. The degree of corneal striae

correlated well with the amount of corneal swelling ($r = 0.83$), which may be helpful in monitoring corneal edema following sleep with rigid lenses.

Corneal hydration recovery was measured from various levels of edema and it was found that the time to return to baseline thickness was related to the initial amount of swelling. Corneal recovery follows a nonlinear time course, with the rate of recovery decreasing as the cornea thins. Further, for any specific level of hydration the rate of recovery was the same regardless of the initial edema induced, suggesting that recovery of the normal cornea is only affected by the current level of edema. During recovery, the corneal thickness always became thinner than baseline (overshoot) for a short period of time. These recovery relationships and "overshoot" phenomenon have not been previously described and may be useful in assessing the function of the endothelial pump mechanism.

CHAPTER 4

IN VIVO ASSESSMENT OF MECHANISMS CONTROLLING CORNEAL HYDRATION

4.1 Summary

The endothelial pump and evaporation components of corneal recovery were studied in the *in vivo* human cornea by inducing corneal swelling using hypoxia and monitoring the subsequent decrease in corneal thickness. Corneal recovery follows a non-linear time course with the rate of recovery decreasing as the cornea thins. Following 60 μm of induced edema, recovery with the eyes open required an average of 2.5 hours to reach baseline corneal thickness, while recovery with the eyes closed took an average of 4.0 hours to reach the normal physiologic corneal swelling (17 μm). Analysis indicates that for open eye recovery from 60 μm of swelling, the endothelial pump provides 20%, while the osmotic thinning caused by tear evaporation contributes 80% of recovery. During recovery, the rate of water evaporation from the anterior corneal surface remained relatively steady at 2.5 $\mu l/cm^2$ x hr. Comparison of measured vs calculated recovery rates during recovery with the eyes closed suggests that the endothelial pump functions at one speed and that the "pump-leak" theory of corneal hydration control is applicable for the human cornea.

4.2 Introduction

Control of corneal hydration is essential for the maintenance of normal transparency [Mishima, 1968]. A compromise in the control mechanisms, due to either a disease process or intervention, can lead to corneal decompensation and loss of vision [Burns et al, 1981; Hoffer, 1979].

Both passive and active mechanisms function in control of corneal hydration. The active control of corneal hydration was first demonstrated by Davson [1955] and Harris & Norquist [1955] in the "temperature reversal" experiments in which excised rabbit cornea hydration increased with cooling and returned to normal upon rewarming. Further studies established the dependence of this phenomenon on metabolism [Dikstein and Maurice, 1972]; and isolated the fluid movement mechanism mainly in the endothelium [Maurice, 1972], the epithelium having only a negligible pumping mechanism [Klyce, 1977]. The endothelial mechanism appears to function by the active transport of bicarbonate [Hodson and Miller, 1976], sodium, and hydrogen ions [Fischbarg and Lim, 1974; Liebovitch and Fischbarg, 1982/83]. The pump is able to move a significant amount of fluid, 6.7 $\mu l/cm^2$ x hr, and appears to function at one speed [Baum et al, 1984]. A description of the model for the endothelial pump mechanism appears in the literature [Maurice, 1984; Fischbarg and Lim, 1984].

Passive factors which oppose the tendency of the stroma to swell [Hedbys and Dohlman, 1963] and help control corneal

thickness were demonstrated by Mishima and Maurice [1961] who showed that fluid evaporation from the anterior corneal surface can have a considerable thinning effect on corneal thickness. This evaporation increases tear osmolarity and contributes to the maintenance of normal hydration [Mishima and Maurice, 1961; Terry and Hill, 1978]. Later work by Mishima & Hedbys [1967] measured the permeability of rabbit corneal epithelium and endothelium to water, showing that these membranes offered some passive resistance to the movement of water into the cornea caused by the stromal swelling pressure.

These active and passive mechanisms provide the basis for the "pump-leak" corneal hydration control theory of Maurice [1962], which states that the fluid leaking into the cornea due to the negative stromal pressure is pumped back out by the active endothelial pump mechanism, to maintain a constant corneal hydration. The passive (hydraulic) leak of fluid into the cornea appears to occur through the intercellular spaces [Baum et al, 1984; Fischbarg et al, 1977], while the flow of fluid out (osmotic) is thought to be across the cell membranes [Liebovitch and Weinbaum, 1981; Fischbarg and Montoreano, 1982]. The flows through the intercellular pathway and across the cells would be equal when the eyes are closed; however, when the eyes are open the flows are unequal, with a transcorneal flow due to normal evaporation at the epithelial surface [Shapiro and Candia, 1973].

The present information on corneal hydration control has primarily been determined from *in vitro* animal studies. There have been few *in vivo* studies which have assessed the hydration control mechanisms for the human cornea. Human studies of endothelial morphology and permeability to fluorescein have been used for relative comparisons between normal and abnormal endotheliums; however, the data from these methods cannot be easily compared to the more fundamental hydration control studies in animals. Further data from human corneas is needed to assess the applicability of the basic animal studies to the *in vivo* human cornea.

In this study, the active and passive mechanisms which control corneal hydration in the *in vivo* human cornea were evaluated by inducing an increase in corneal hydration and then monitoring the hydrodynamic response of the cornea during recovery under specific environmental conditions. The relative contribution of the endothelial pump and evaporation components of corneal recovery were determined. Change in endothelial pump rate in response to increased corneal hydration and the applicability of the "pump-leak" theory of corneal hydration control were assessed for the human cornea. The results suggest that this technique may provide an *in vivo* test to assess endothelial function.

4.3 Materials and Methods

Subjects

Ten subjects, (one woman, nine men; mean age 26.7 $\pm$ 4.8 years, range 23 to 37 years) who were free of ocular disease and had no prior contact lens experience (unadapted) participated in the study. Informed consent was obtained from each subject. A summary of relevant ocular parameters is listed in Table 4.1.

Corneal Swelling

To increase corneal hydration the anterior corneal surface was exposed to hypoxia by having the subjects wear a Bausch & Lomb U4 hydrogel lens (Bausch & Lomb Inc, Rochester, NY) with an oxygen transmissibility (Dk/L) of 10.5×10^{-9} (cm/sec)(ml O_2/ml x mmHg) for 3 hours with the eyes closed. This contact lens reduced the level of oxygen at the corneal surface to approximately 8 mmHg [O'Neal et al, 1984], below the minimum oxygen tension needed for normal metabolism [Mandell and Farrell, 1980]. Corneal hypoxia results in increased stromal lactate, which creates an osmotic imbalance and causes corneal swelling [Klyce, 1981].

Table 4.1. Summary of selected ocular parameters for the 10 subjects.

	Mean	SD	Range
Age (years)	26.7	4.8	23 to 37
Corneal thickness (μm)	509	24	472 to 551
Sphere ref. error (D)	-0.54	1.86	+0.75 to -6.25
Cylinder ref. error (D)	-0.36	0.48	0 to -1.75
Horiz. Keratometry (D)	43.26	1.50	39.75 to 46.00
Corneal toricity (D)	0.60	0.47	0.37 against to 1.50 with

Procedure

Changes in corneal hydration were monitored by measuring central corneal thickness [Hedbys and Mishima, 1966] using a Haag-Streit pachometer (Haag-Streit, Waldwick, NJ) adapted to a biomicroscope and connected to an electronic digital pachometer unit. The pachometer design was similar to that described by Holden et al [1982], with the angle between the slit beam and ocular set at 75 degrees to increase sensitivity. Each measurement included 10 readings with a standard deviation of $\pm$ 4.0 microns.

Baseline corneal thickness was measured at least 3 hours after awakening to eliminate any influence sleep may have on thickness [Mertz, 1980]. The hydrogel lens was worn on the right eye only, with the left eye serving as a control. After removing the lens, recovery of corneal thickness was monitored for 4 hours. To maintain a constant environment, the subjects remained in the test area throughout each session.

Experiment 1. When the eyes are closed (eg. sleep) the corneal thickness increases, which is thought to be due to a shift in tear tonicity [Mishima and Maurice, 1961; Chan and Mandell, 1975]. The level of normal physiologic closed eye corneal swelling for these subjects was measured after 3 & 6 hours of eye closure when no lens was worn.

Experiment 2. Corneal recovery from induced swelling was monitored with the eyes open, after removing the test lens, by measuring corneal thickness every 15 minutes for the first hour and each one-half hour for the next 3 hours. On a

separate day, the same subjects again had corneal edema induced using the test lens; however, in this session recovery after lens removal was monitored with the eyes closed. Corneal thickness measurements were made for 4 hours at 1 hour intervals with the eye open for 30 seconds to allow measurement.

Experiment 3. The difference in recovery rates between opening the eye every hour (Exp. 2) for 3 hours and after 3 hours with the eye remaining closed was assessed. There was no difference for these two conditions, indicating that opening the eye for a brief period (30 seconds) every hour did not affect the time course of recovery with the eyes closed.

Experiment 4. Corneal recovery with the eyes closed eliminates the effect of evaporation on recovery; however, the decreased level of oxygen (PO_2 55 mmHg) available from the conjunctiva [Efron and Carney, 1979] during eye closure might affect the rate of recovery. These effects on recovery were evaluated using 6 additional subjects. Corneal swelling was induced in both eyes and following lens removal, recovery was monitored with one eye closed and the contralateral eye open, while the subject stayed in a room having 100% humidity and normal (PO_2 155 mmHg) oxygen tension.

Experiment 5. The effect of hypoxia (and presumeably lactate [Klyce, 1981]) on the rate of recovery was assessed by inducing the same level of corneal swelling (60 μm) under two different conditions. First, edema was induced with the Bausch & Lomb U4 hydrogel lens worn during eye closure; and

secondly, by wearing a thick hydrogel lens (38% H_2O, 0.65 mm thick, $Dk/L = 0.2 \times 10^{-9}$ (cm/sec)(ml O_2/ml x mmHg) with the eyes open. This paradigm was chosen since it is believed that corneal swelling induced during eye closure includes both a hypoxic and osmotic component [Fatt and Chaston, 1981], while swelling induced with the eyes open is primarily due to hypoxia. Although lactate levels were not directly measured, it may be assumed that more lactate is in the stroma for hypoxic swelling induced with the eyes open than when the same level of swelling is induced with the eyes closed. If recovery rates from these two hypoxia conditions are the same, then presumably hypoxia and the excess stromal lactate does not affect the dehydration mechanism.

Figure 4.1 shows the decrease in central corneal swelling over time after lens removal (recovery) with the eyes open following edema induced with hypoxia created both with the eyes closed and with the eyes open. There was no difference in the time course of recovery from the same level of edema induced with the eyes closed or open, indicating that inducing edema using hypoxic stress (presumeably due to increased stromal lactate) has no effect on the recovery mechanism.

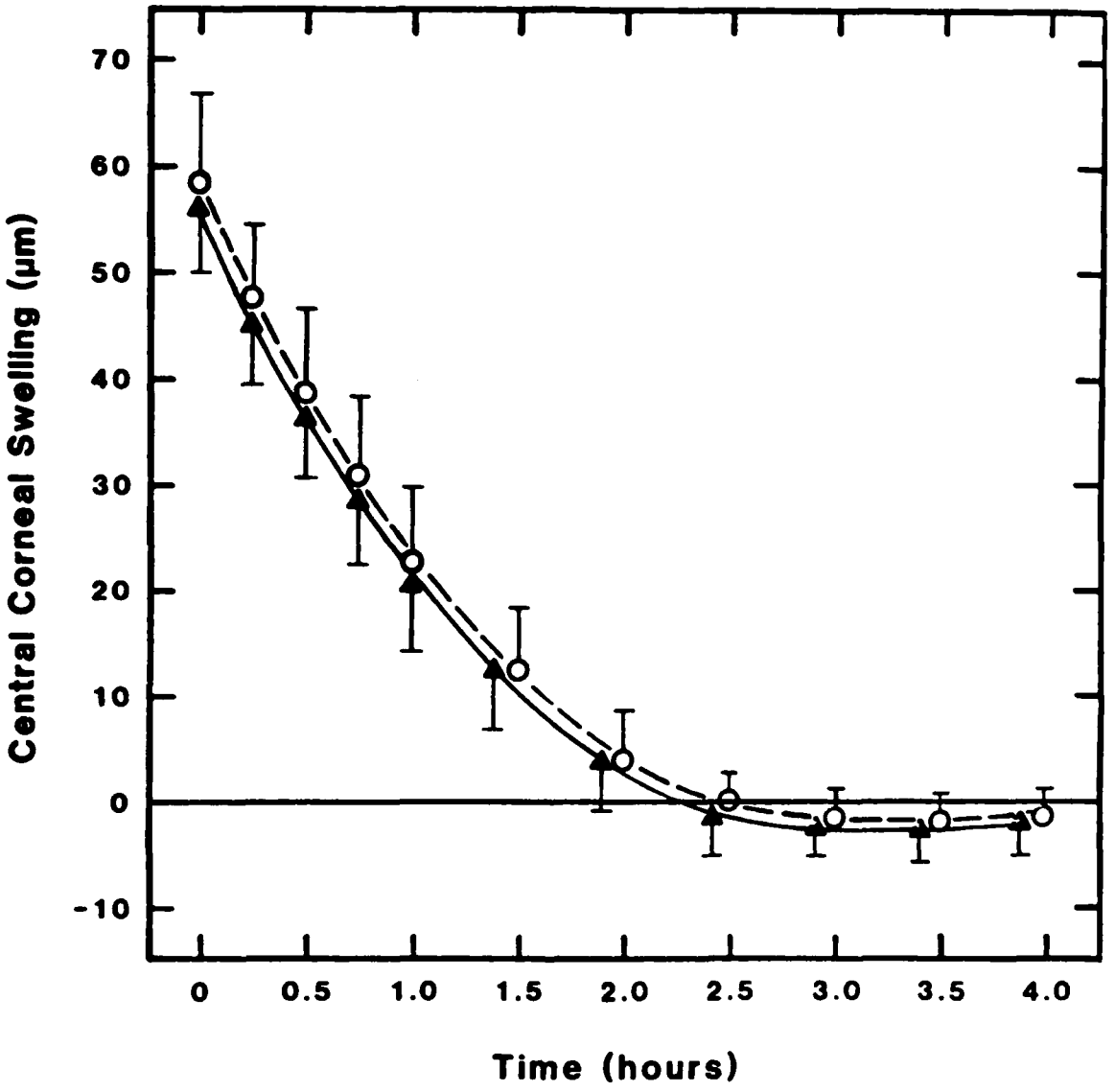

Figure 4.1. Mean decrease in central corneal swelling vs time for 10 subjects during recovery with the eyes open. Corneal swelling was induced with the eyes open (filled triangles) and closed (open circles). Error bars equal ± 1 SD.

Recovery Due To The Endothelial Pump

Calculation of the rate of fluid flow out of the cornea due to the endothelial pump was made based upon the calculated stromal swelling pressure and a steady state endothelial pump rate, using the following formula:

$$\text{Recovery flow} = \frac{\text{IPswell} - \text{IPclsd}}{\text{IPclsd}} \times \text{Pump Rate} \qquad (4.1)$$

where IPswell & IPclsd are the stromal imbibition pressures at the mean swelled and closed eye corneal thicknesses, respectively; and the endothelial pump rate is taken as 6.7 $\mu l/cm^2$ x hr, as reported in the rabbit by Baum et al [1984] for the rate of fluid transport at zero applied transtissue hydrostatic pressure.

This formula states that the amount of decrease in the fluid leak into the cornea equals the net fluid flow out due to the pump and can be calculated from the fractional change in imbibition pressure times the endothelial pump rate. This calculation includes the following assumptions:

1. The water leakage rate into the cornea decreases as the cornea swells and this decrease is directly proportional to the reduction in swelling pressure that occurs with greater stromal hydration [Hedbys and Dohlman, 1963]. The difference in this decreased leak and the endothelial pump rate is the rate at which fluid should flow out of the cornea due only to the endothelial pump.

2. The steady state endothelial pump rate is the fluid flow out of the cornea which equals the fluid leakage in due to the stromal imbibition pressure (IP), to maintain the cornea at the closed eye thickness [Shapiro and Candia, 1973]. The difference between the closed and open eye corneal thickness is due to osmotic thinning caused by tear evaporation [Mishima and Maurice, 1961; Terry and Hill, 1978], with the cornea receiving adequate oxygen to prevent hypoxia during eye closure [Mandell and Farrell, 1980; Efron and Carney, 1979].

The imbibition pressure (IP) is the stromal swelling pressure (SP) minus the hydrostatic compressive effect of the intraocular pressure (IOP = 18 mmHg) [Hedbys et al, 1963; Friedman, 1973]. The swelling pressure was calculated using the relationships of corneal thickness to hydration, and then hydration to stromal swelling pressure, in which:

$$H = (7.0 \times CT) - 0.64 \qquad (4.2)$$

and

$$SP = 1{,}555\ e^{-H} \qquad (4.3)$$

where H is gram water/gram dry weight and CT is corneal thickness in mm [Hedbys and Mishima, 1966]. The swelling pressure equation shown was derived by replotting the data points of Hedbys & Dohlman [1963] and Fatt & Hedbys [1970] for human stroma onto a semilogarithmic scale to demonstrate the linear relationship ($r = 0.965$). For our group mean physiologic closed eye corneal thickness of 526 µm, the calculated imbibition pressure is IPclsd = 56.2 mmHg.

The rate of water leakage into the cornea is also related to the hydraulic conductivity (Lp) of the membranes and the pressure gradient across them. However, a number of Lp values have been reported for the corneal endothelium, with large differences between the hydraulically, osmotically, and mathematically determined LP values [Mishima and Hedbys, 1967; Fischbarg et al, 1977; Klyce and Russell, 1979]. Therefore, as suggested by Burns et al [1981], the rate of water leakage into the cornea was not calculated using the membrane hydraulic conductivity. To eliminate this factor from the calculations, the hydraulic conductivity values of all subjects in this study are taken as equal and normal.

4.4 Results

Figure 4.2 compares the decrease in central corneal swelling over time (recovery) with the eyes closed from 60.1 $\pm$ 4.2 µm to recovery with the eyes open from 59.1 $\pm$ 4.4 µm of induced swelling. Corneal recovery with the eyes either open or closed follows a nonlinear time course with the rate of recovery decreasing as the cornea thins. When the eyes remained closed during recovery, the time to reach the normal physiologic closed eye swelling, 17.2 $\pm$ 4.1 µm (dotted line), was approximately 4.0 hours. When recovery was monitored with the eyes open, the time to reach this same level of edema (17 µm) was 1.25 hours, while the baseline corneal thickness was reached in 2.5 hours. During recovery with the eyes open, the cornea became thinner than baseline (overshoot) by approximately 2-3 m for 30 to 90 minutes during the last 1.5 hour of measurement. (See Appendixes 6 and 7 for the individual recovery data).

The recovery curves were fitted by the method of least squares to 3rd order polynomial equations:

$$CS = 60.1 - 18.8\ t + 2.1\ t^2 - 0.02\ t^3 \qquad (4.4)$$

for recovery with the eyes closed ($n = 50$, $r = 0.9704$), and

$$CS = 59.1 - 46.4\ t + 11.4\ t^2 - 0.9\ t^3 \qquad (4.5)$$

for recovery with the eyes open ($n = 110$, $r = 0.9881$);

where CS is the corneal swelling in µm, and t is the time in hours since lens removal (recovery).

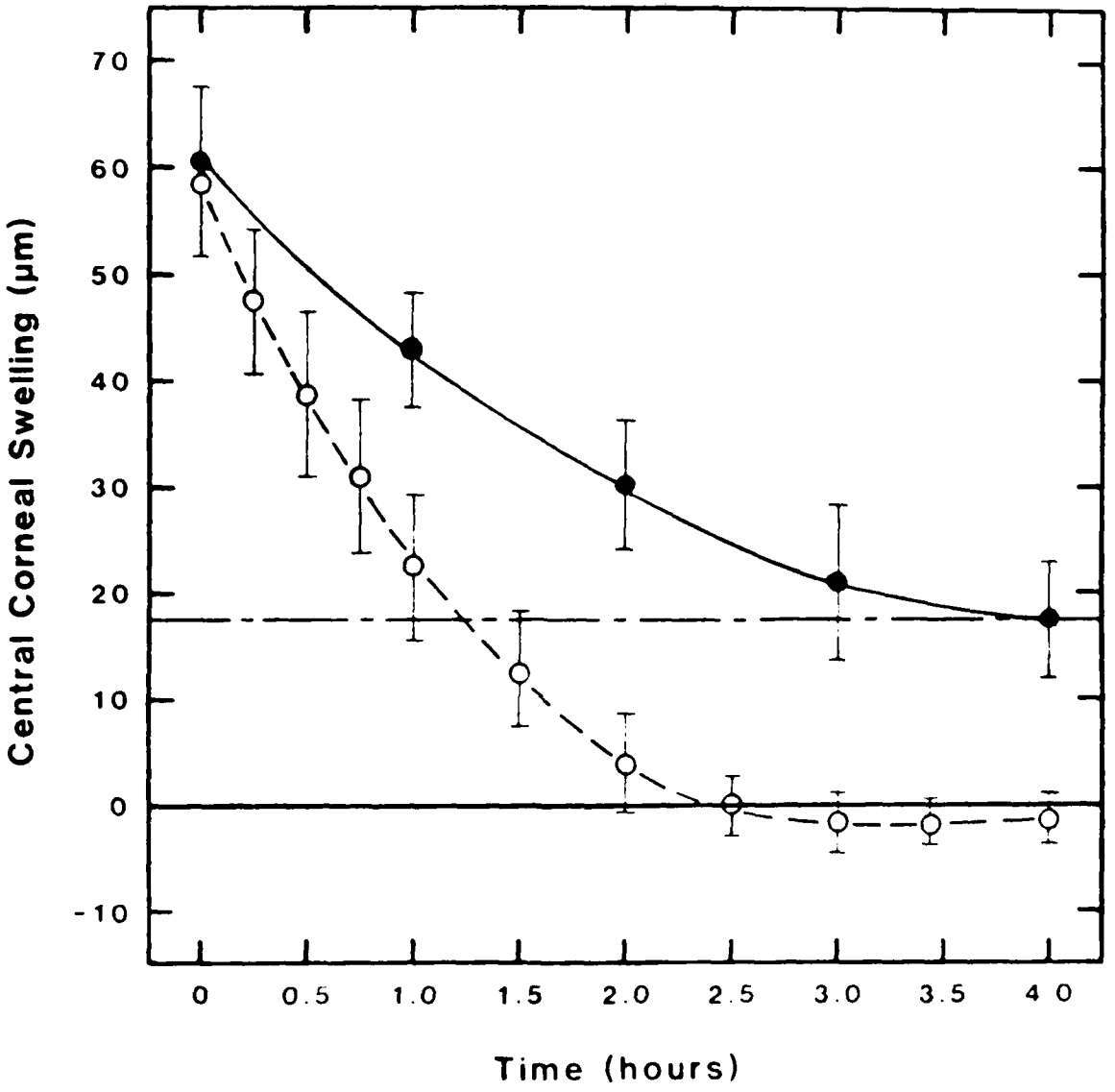

Figure 4.2. Mean decrease in central corneal swelling vs time for 10 subjects during recovery with the eyes open (open circles) and closed (filled circles) following corneal swelling induced with the eyes closed. Error bars equal ± 1 SD and lines were fitted by polynomial equation.

Dependency of the data was not formally addressed using statistical procedures; however, it appears to be minimal.

The influence of the lower level of oxygen (PO_2 55 mmHg) [Efron and Carney, 1979] on recovery with the eyes closed was assessed on 6 additional subjects. These additional subjects showed similar open eye recovery compared to the main subject group. Figure 4.3 shows the decrease in corneal swelling for the eye remaining closed and the contralateral eye which was open and exposed to a 100% humidified environment (PO_2 155 mmHg). Differences in the time course of recovery for these two conditions were small (approximately 1 μm/hr). These data indicate that the slower recovery rate when the eyes are closed as compared to when the eyes are open, is not influenced by the reduction in oxygen that occurs with eye closure; but rather is a result of the difference between recovery in a 100% humidified and in a normal (60%) humidity open eye environment. (See Appendixes 8 - 10 for the individual recovery data of these additional subjects).

The difference in the slower "leak" for the swelled cornea and the endothelial pump rate, is the rate at which fluid should move out of the cornea due only to the endothelial pump, when evaporation is eliminated. Comparison of the calculated fluid flow out of the cornea due to a steady state pump (see 4.3 Methods) and the measured flow, obtained using the 1st derivative of equation 4.4 from Figure 4.2, during recovery with the eyes closed (ie. without evaporation) is shown in Table 4.2.

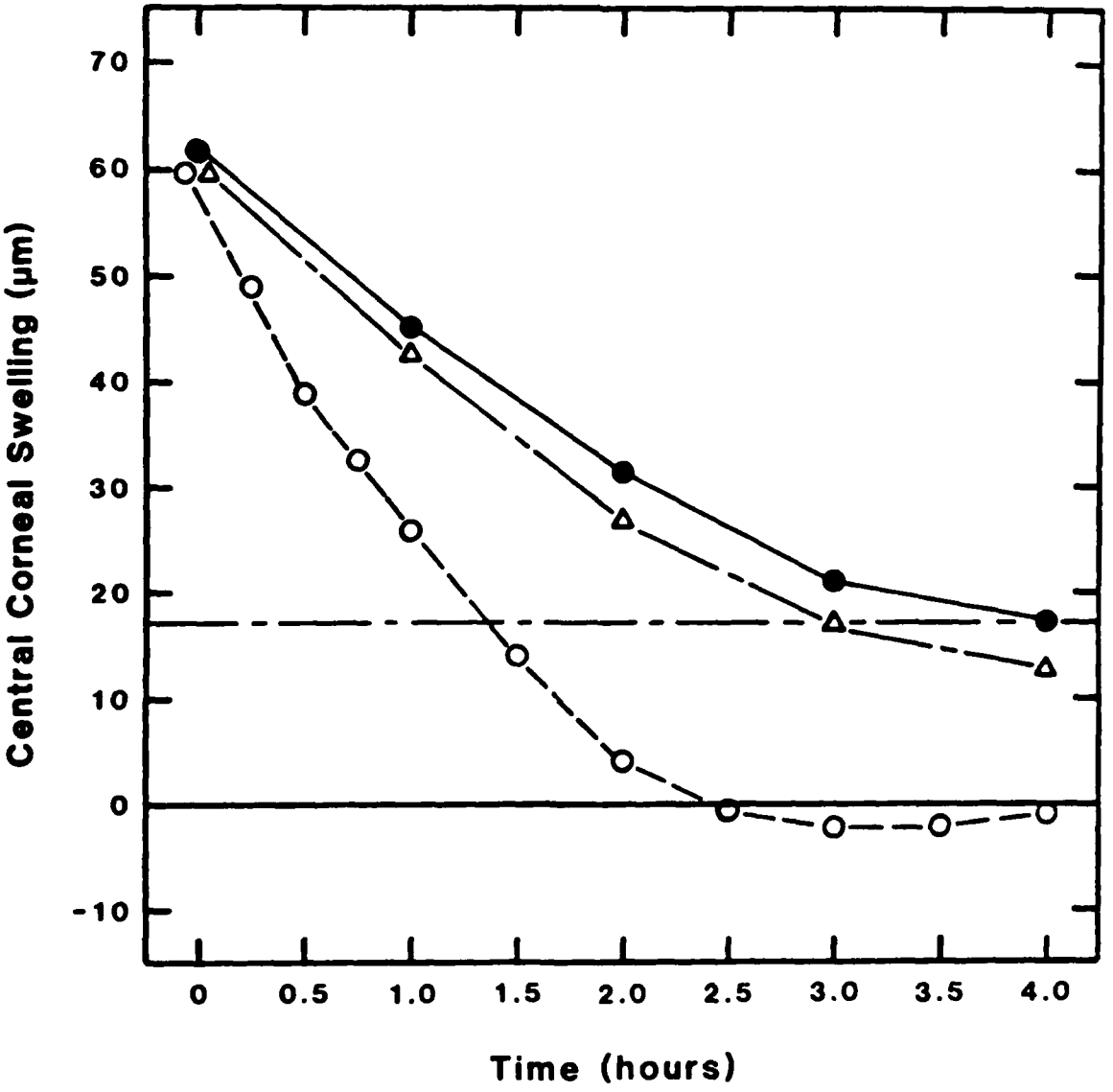

Figure 4.3. Mean decrease in central corneal swelling vs time for 6 subjects during recovery with the eyes closed (filled circles), and with the eyes open in 100% humidity (open triangles) and 60% humidity (open circles) environments. Corneal swelling was induced with the eyes closed. The standard deviation for each data point ranged from 1.1 to 3.7 µm, with mean SD = 2.2 $\pm$ 0.7 µm.

Table 4.2. Measured vs calculated corneal recovery due to the endothelial pump. Recovery due to pump = Fractional decrease in Imbibition Pressure (IP) x Pump Rate.

Pump rate = 6.7 $\mu l/cm^2$ x hr.

Recovery Time (hrs)	Corneal Swelling (μm)	Calculated Imbibition Pressure (mmHg)	Fractional Decrease in IP	Recovery due to PUMP (Net Outflow) Calculated ($\mu l/cm^2$ x hr)	Measured
0	60.1	36.9	0.34	2.2	1.9
1.0	43.4	43.8	0.22	1.5	1.5
2.0	30.7	49.5	0.12	0.8	1.0
3.0	22.1	53.7	0.05	0.4	0.6
4.0	17.2	56.2	0	0	0.2

There is close agreement between the calculated and measured fluid flow out of the cornea throughout the entire recovery phase. The slight difference in flows, 0.2 $\mu l/cm^2$ x hr (2 μm/hr rate of recovery), is within the experimental error of pachometric measurement. This finding suggests that recovery with the eyes closed is due to the endothelial pump and that the pump appears to function at one speed.

This finding also indicates that the rate of recovery due only to the endothelial pump (during eye closure) is directly related to the amount of swelling remaining. Therefore, by comparing the recovery rates for the open and closed eye at corresponding levels of swelling, it is possible to separate the evaporation and pump components of recovery. The rates of recovery with the eyes open or closed were calculated using the 1st derivative of the equations derived from the data in Figure 4.2. The recovery rates were calculated at 15 minute intervals during recovery with the eyes open, and at corresponding levels of swelling remaining during closed eye recovery, and are shown in the upper and lower curves in Figure 4.4. For example, after 30 minutes of recovery with the eyes open, the amount of swelling remaining is 38 μm and the rate of recovery is 36 μm/hr; while for recovery with the eyes closed, the recovery rate is only 12 μm/hr at this same 38 μm level of remaining edema.

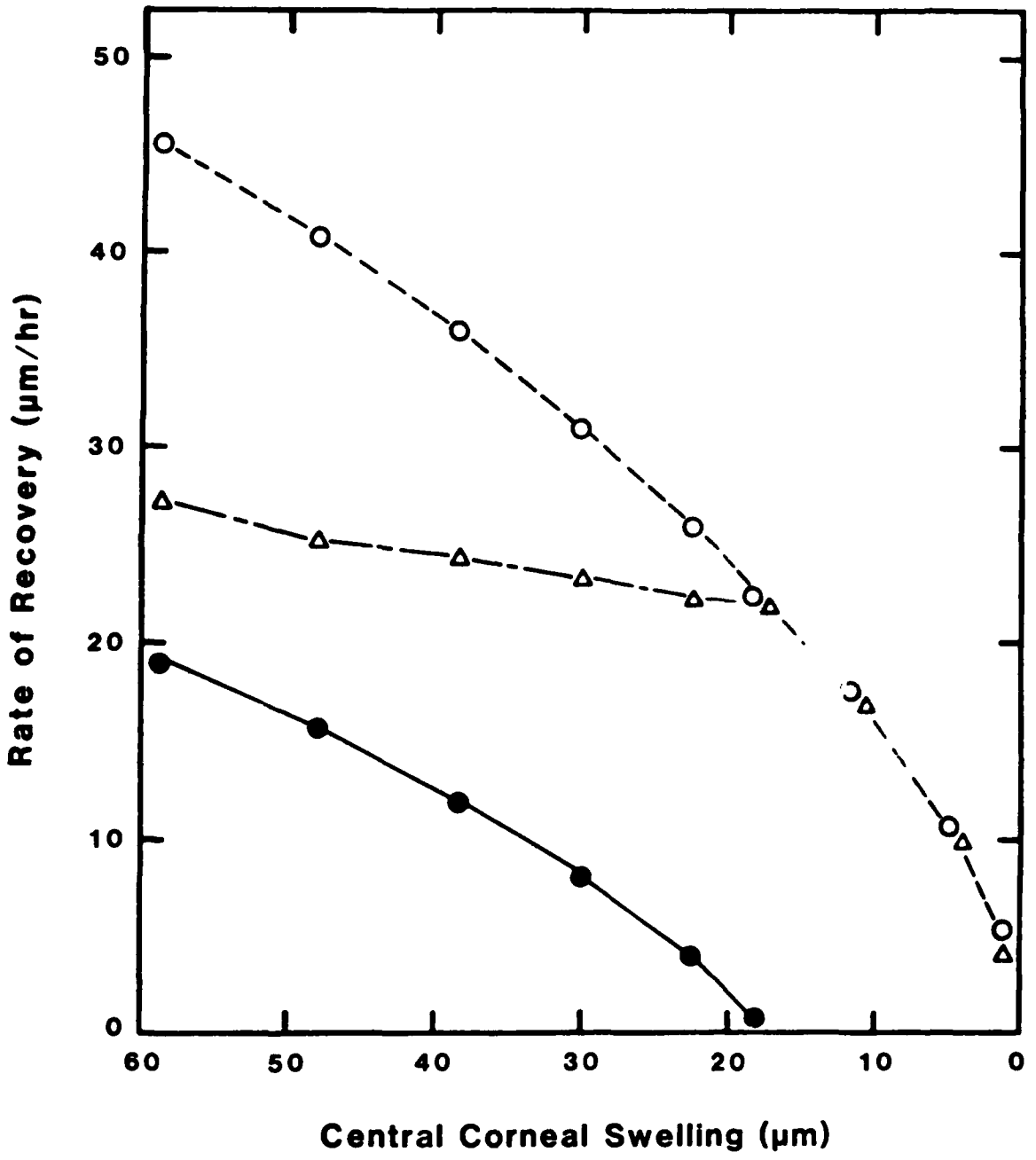

Figure 4.4. Rate of recovery vs mean central corneal swelling (remaining edema) with the eyes open (open circles) and closed (filled circles). The evaporation component of recovery (open triangles) is the difference between the open and closed eye rates of recovery at corresponding levels of swelling calculated for the amount of swelling remaining at 15 minute intervals during open eye recovery.

This analysis provides a comparison of recovery rates for corneas at the same level of stromal fluid pressure during both recovery conditions. At any level of swelling remaining, the rate of recovery with the eyes open was faster than that with the eyes closed. Subtraction of the rate of recovery with the eyes closed from the recovery rate with the eyes open, provides quantification of the osmotic effect of tear evaporation on recovery at any level of swelling, middle curve in Figure 4.4. These calculations show that the evaporation component of recovery remained steady at approximately 25 μm/hr until the normal level of closed eye swelling (17 μm) was reached; at this point the rate of recovery decreased as the cornea approached baseline thickness.

The recovery rates for the endothelial pump (ie. closed eye) in Figure 4.4 were used to calculate the decrease in corneal swelling during open eye recovery that is due to the pump, with the remainder due to evaporation. Each data point in Figure 4.4 corresponds to the rate of recovery at the level of swelling remaining at 15 minute intervals during open eye recovery. The average rate of recovery for each interval was used to compute the corneal thickness decrease due to the pump. For example, the initial closed eye recovery rate at 60 μm of swelling was 19 μm/hr, while after 15 minutes of open eye recovery the corneal swelling had decreased to 48 μm, with the corresponding recovery rate due to the pump now 16 μm/hr. This gives an average recovery rate of 17.5 μm/hr and 4.4 μm of recovery due to the pump during the first 15 minutes of

open eye recovery.

Repeating this process for each 15 minute period until the normal closed eye corneal swelling is reached (at which point the pump no longer contributes to recovery with the eyes open), gives an additional 3.5, 2.6, 1.8, and 0.7 μm of recovery due to the pump. These calculations indicate that the endothelial pump contributed 13 μm (20%), while evaporation contributed 47 μm (80%) of the total recovery from this (60 μm) level of swelling. A comparison of the relative contribution of the endothelial pump and evaporation to recovery with the eyes open from 60 μm of induced swelling is shown in Figure 4.5.

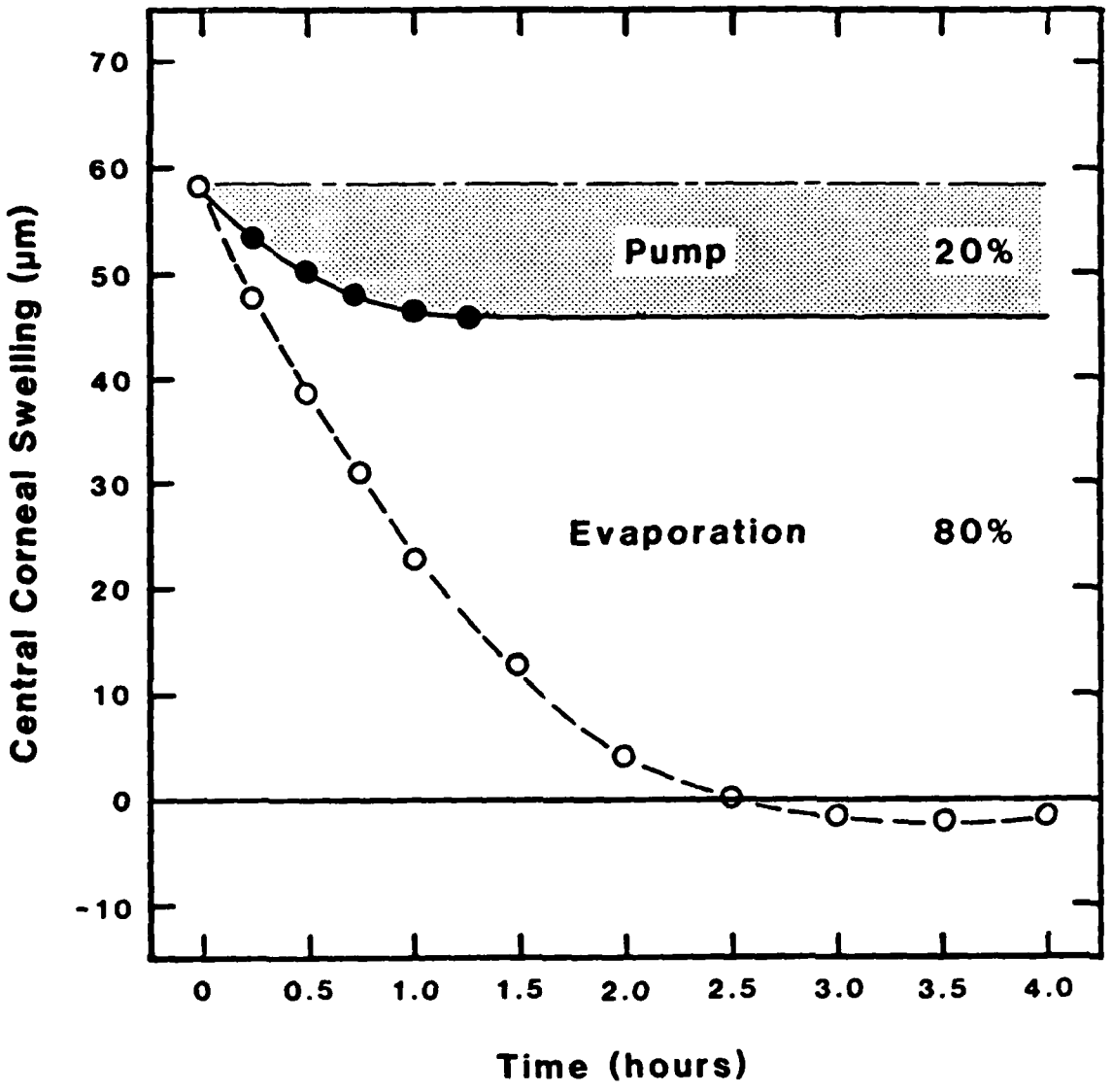

Figure 4.5. Comparison of the relative contribution of the endothelial pump (shaded area) and osmotic thinning caused by tear evaporation to the mean decrease in central corneal swelling vs time for recovery with the eyes open from an initial 60 µm of corneal swelling .

4.5 Discussion

Corneal recovery from induced edema follows a nonlinear time course, with the rate of recovery decreasing as the cornea thins. The rate of recovery was considerably faster with the eyes open than with the eyes closed. Analysis of these recovery rates suggest that recovery from edema with the eyes open includes the effect of tear evaporation from the anterior corneal surface and the endothelial pump, and that when the eyes are closed the endothelial pump is primarily responsible for removing the excess fluid.

There was no difference in the rate of recovery with the eyes open following edema induced with the eyes open or closed. This finding indicates that the use of hypoxic stress to induce corneal edema does not affect the subsequent corneal recovery. The time course of recovery was similar for the open eye exposed to a 100% humidity environment (ie. no evaporation, PO_2 155 mmHg) compared to the closed eye; which indicates that the normal decrease in oxygen with the eyes closed (PO_2 55 mmHg) does not affect recovery. This finding also indicates that the differences in recovery rates under closed and open eye conditions is due to the environment humidities (100% humidity with the eyes closed vs 60% humidity with the eyes open), which would result in different tear film osmolarities.

Analysis of these data indicates that for open eye recovery from 60 μm of edema, the endothelial pump provided

approximately 20% of the recovery, while the remaining 80% was contributed by the osmotic thinning effect of tear evaporation. However, the contribution of each component is related to the initial level of swelling, since recovery from the first 17 μm of swelling is entirely due to evaporation in our analysis. For example, the calculated contribution of the endothelial pump to recovery is also 30, 26, and 13% from 90, 75, and 40 μm of initial corneal swelling, respectively. This suggests that for the normal cornea that undergoes edema, the osmotic thinning effect of tear evaporation is an important and substantial function in returning the cornea to normal hydration.

The difference between the recovery rates (open & closed eye) at corresponding levels of swelling were used to calculate the rate of recovery due to the osmotic effect of tear evaporation. These calculations indicate that the net flow of fluid out of the cornea due to evaporation remained constant at approximately 2.5 $\mu l/cm^2$ x hr until the cornea approached the normal physiologic closed eye swelling (17 μm), at which point the net flow out decreases. This fluid flow value for the effect of evaporation is in agreement with that found by Mishima & Maurice [1961] for the rabbit cornea.

It was also noted that the cornea became thinner (overshoot) that the baseline corneal thickness during the last 1.5 hour of open eye recovery. There is normally a transcorneal fluid flow due to evaporation at the epithelial surface [Shapiro and Candia, 1973], and the transient

overshoot observed during recovery might be due to a disparity between the rate of water removal from the anterior stroma due to evaporation and the fluid movement inward through the endothelium. The cornea might be expected to be thinner than normal until the normal transcorneal flow is reestablished.

To assess changes in endothelial pump rate for these subjects, comparison was made between the measured and calculated fluid flow out of the cornea attributable to the pump. This flow is based on the calculated fractional decrease in stromal imbibition pressure that occurs with increased corneal hydration and a reported baseline endothelial pump rate, 6.7 $\mu l/cm^2$ x hr, for the rabbit endothelium at zero transtissue hydrostatic pressure. The baseline pump rate may also be obtained by extrapolation on the data in Figure 2 of Fischbarg et al [1977], which yields a value of about 8 $\mu l/cm^2$ x hr and gives calculated recovery rates similar to those reported. The degree of correspondence between the measured and calculated fluid outflows is dependent upon the limitations of the assumptions and the apparent pump rate employed in the calculation; however, within the variance of the pachometer measurement technique our conclusions appear to be appropriate.

Comparison of the calculated fluid flow out due to a steady state endothelial pump rate to the measured flow during recovery with the eyes closed (ie. when evaporation was completely eliminated) showed good correspondence (within 2 $\mu m/hr$ recovery rate) throughout the entire recovery phase.

This finding suggests that the endothelial pump functions at one speed regardless of the level of corneal hydration, which is in agreement with the results of Baum et al [1984] for the rabbit cornea. These recovery data for the in vivo human cornea also fit the rate of recovery predicted by the "pump-leak" theory of corneal hydration control.

These findings indicate that inducing corneal edema using hypoxic stress and monitoring the subsequent recovery may provide a clinical test to assess endothelial function. Further studies of this method to provide a clinical test of endothelial function are indicated.

CHAPTER 5

DECREASED ENDOTHELIAL PUMP FUNCTION WITH AGING

5.1 Summary

Endothelial function may be affected by the endothelial cell loss and increased variability in cell shape and size (polymegathism) that accompany normal aging. Endothelial function can be evaluated by monitoring corneal hydration recovery following hypoxic stress. Corneal recovery and endothelial morphology were compared between a group of younger (mean age = 26.7 yrs) and older (mean age = 65.7 yrs) subjects with normal corneas. Edema (60 μm) was induced with hydrogel lenses worn with the eyes closed. Following lens removal, the decrease in corneal thickness was monitored for 4 hours with one eye open while the contralateral eye remained closed. For both age groups, corneal recovery followed a nonlinear time course. The open eye required 2.5 hours and 3.0 hours to return to baseline for the younger and older age groups, respectively. Recovery during eye closure took 3.5 hours to reach the normal closed eye level for the younger subjects and was not complete at 4 hours for the older subjects. Recovery rates were significantly slower for the older vs younger subjects during the first 2 hours of closed eye recovery, 10.5 vs 15.0 μm/hr, and for the initial 1 hour of open eye recovery, 26.5 vs 35.6 μm/hr. The rate of

recovery was negatively correlated with the coefficient of variation in cell area, $r = -0.62$ and -0.69 ($p < 0.01$), for both closed and open eye recovery, respectively. These data suggest that endothelial pump function decreases approximately 10% by age 65 and indicates a possible link between endothelial morphology and function.

5.2 Introduction

Endothelial cell loss and increased variability in cell shape (pleomorphism) and size (polymegathism) accompany normal aging [Laing et al, 1976; Laule et al, 1978]. Since aging alters many bodily functions, it might be expected that these endothelial cell changes would affect the ability of the endothelium to maintain normal corneal hydration [Mishima, 1982]. Although a low cell density can lead to corneal decompensation [Hoffer, 1979; Bodereau et al, 1983], the degree of cell loss accompanying aging apparently does not alter corneal hydration [Bourne and Kaufman, 1976]. Similarly, studies involving older patients have reported no correlation between the postoperative endothelial cell density and degree of corneal swelling following intraocular lens implant surgery [Rao et al, 1978; Olsen, 1980]. Recently, however, a relationship was found between this postoperative corneal edema the degree of polymegathism prior to surgery [Rao et al, 1984], suggesting that endothelial polymegathism and function may be related.

The endothelium has both an active pump and passive fluid barrier functions [Maurice, 1984]. A recent study on the relationship between aging and the endothelial barrier function found no correlation between age and the endothelial permeability to fluorescein [Bourne et al, 1984]. Indeed, it has been reported that younger subjects have a higher endothelial fluorescein permeability compared to older

subjects [Sawa et al, 1983]. A decreased endothelial permeability to fluorescein was found to correlate with an increase in cell size (ie. lower cell density), but not with the variation in cell size of transplanted corneas [Bourne and Brubaker, 1983]. These studies would seem to indicate that the endothelial barrier function is not compromised by either a moderate loss of endothelial cells or increased cell polymegathism. This suggests that it is the other endothelial function, the active pump, that may be affected by morphological changes in the endothelium. There have been no studies, however, to determine if the endothelial pump is affected during aging or is correlated with endothelial morphology.

In vivo endothelial function can be assessed by measuring changes in corneal hydration following hypoxic stress [O'Neal and Polse, 1985]. Corneal recovery occured at approximately the rate predicted for the endothelial pump, suggesting that this method provides a direct evaluation of endothelial pump function. The time course of hydration recovery was determined for a group of young subjects with normal corneas. Comparison of these recovery data to those of older subjects with normal corneas also, may allow assessment of age-related changes in endothelial function. Comparison of corneal recovery to endothelial cell analysis should provide information on the correlation of endothelial function to morphology.

In this study, the time course of corneal hydration recovery following hypoxic stress was measured on a group of older subjects and compared to the recovery profiles of the younger subjects. Endothelial photomicrographs were evaluated to determine if there is a relationship between the corneal hydration recovery response and endothelial morphology. These data suggest that endothelial pump function decreases with aging and indicates a possible link between endothelial morphology and function.

5.3 Materials and Methods

Subjects

Ten subjects, (7 women, 3 men; mean age 65.7 $\pm$ 2.8 years, range 62 to 71 years) who were free of ocular disease and were not contact lens wearers participated in the study. Informed consent was given by each subject. A summary of relevant ocular parameters is listed in Table 5.1. The younger group, which had been measured previously under the same conditions, included ten subjects, (1 woman, 9 men; mean age 26.7 $\pm$ 4.8 years, range 23 to 37 years) with similar ocular parameters (see Table 4.1).

Corneal Swelling

Corneal hydration was increased by exposing the anterior corneal surface to a hypoxic environment. Subjects wore a piggyback combination of a B4 and U4 Bausch & Lomb hydrogel lens with a total thickness of 0.20 mm and an oxygen transmissibility (Dk/L) of 4.5×10^{-9} (cm/sec) (ml O_2/ml x mmHg). This contact lens combination reduced the oxygen level at the lens-cornea interface to approximately 4 mmHg [O'Neal et al, 1984], which is below the oxygen level required to prevent corneal swelling [Polse and Mandell, 1970; Mandell and Farrell, 1980].

Table 5.1. Summary of selected ocular parameters for the 10 subjects.

	Mean	SD	Range
Age (years)	65.7	2.8	62 to 71
Corneal thickness (μm)	510	31	463 to 571
Sphere ref. error (D)	+2.01	1.64	+5.50 to -0.25
Cylinder ref. error (D)	-0.43	0.43	0 to -1.25
Horiz. Keratometry (D)	44.11	0.92	43.00 to 45.75
Corneal toricity (D)	0.50	0.24	0.75 against to 1.00 with

Endothelial Cell Morphology

Endothelial photomicrographs were obtained prior to lens insertion using a Nikon Non-Contact Endothelial Microscope and Kodak Ektachrome 200 slide film. Each photomicrograph was projected onto a screen at known magnification, and only cells in which the complete cell margin could be visualized were then traced onto paper. From this outline each cell boundary was again traced on a Bausch & Lomb Hipad Digitizer table connected to a Nova 1230 Computer. For each tracing computer analysis determined the mean and standard deviation of cell area, from which the coefficient of variation in cell area (SD/Mean cell area x 100) was calculated. This coefficient expresses the standard deviation as a percentage of the mean cell area, and provides an index of the variation of cell size (polymegathism) [Burns et al,1981; Rao et al, 1984]. The closer the value to zero, the more uniform the cell size; and the closer the value to 100, the greater the variation in cell size.

Procedure

Corneal hydration changes were monitored by measuring central corneal thickness [Hedbys and Mishima, 1966] using an optical pachometer which had been modified to increase accuracy. Each measurement included 10 readings with a standard deviation of $\pm$ 4.0 microns. Baseline corneal thickness was measured at least 3 hours after awakening to eliminate any influence sleep may have on thickness [Mertz,

1980]. The hydrogel lens combination was then inserted in each eye and worn for 1.5 hours with both eyes closed. This procedure resulted in approximately 60 μm of corneal swelling. The lenses were then removed and corneal thickness was monitored for 4 hours. During the recovery period one eye was kept open and measurements were made every 30 minutes, while the contralateral eye remained closed (except for brief 30 seconds measurement every hour). In a separate session, the normal physiologic closed eye swelling (23.3 $\pm$ 5.1 μm) was measured after 3 hours of eye closure when no lens was worn. The procedures used for these older subjects were the same as those used for the younger subjects measured previously. The instruments and environmental conditions were identical for the two groups.

5.4 Results

Figure 5.1 shows the progressive decrease in central corneal swelling (recovery) over the 4 hour time period for the open and closed eye conditions from 58.6 $\pm$ 4.8 μm of induced edema. The recovery was non-linear, with the rate of recovery decreasing as the cornea thins. For the open eye, the cornea returned to baseline thickness in about 3.0 hours; while with the eyes closed, the cornea did not reach the normal physiologic closed eye edema (dotted line) in the 4 hour recovery period. (See Appendixes 11 and 12 for the individual recovery data).

The recovery curves were fitted by the method of least squares to 3rd order polynomial equations:

$$CS\ (closed) = 58.8 - 11.9\ t + 0.3\ t^2 + 0.1\ t^3 \qquad (5.1)$$

for recovery with the eyes closed (n = 50, r = 0.9234), and

$$CS\ (open) = 58.8 - 32.0\ t + 8.2\ t^2 + 0.09\ t^3 \qquad (5.2)$$

for recovery with the eyes open (n = 110, r = 0.8671);

where CS is the corneal swelling in μm, and t is the time in hours since lens removal (recovery).

Comparison of the time course of closed eye recovery for these older subjects to the younger subjects is shown in Figure 5.2. The younger age group recovers faster compared to the older age group from a similar (60 μm) level of initial swelling.

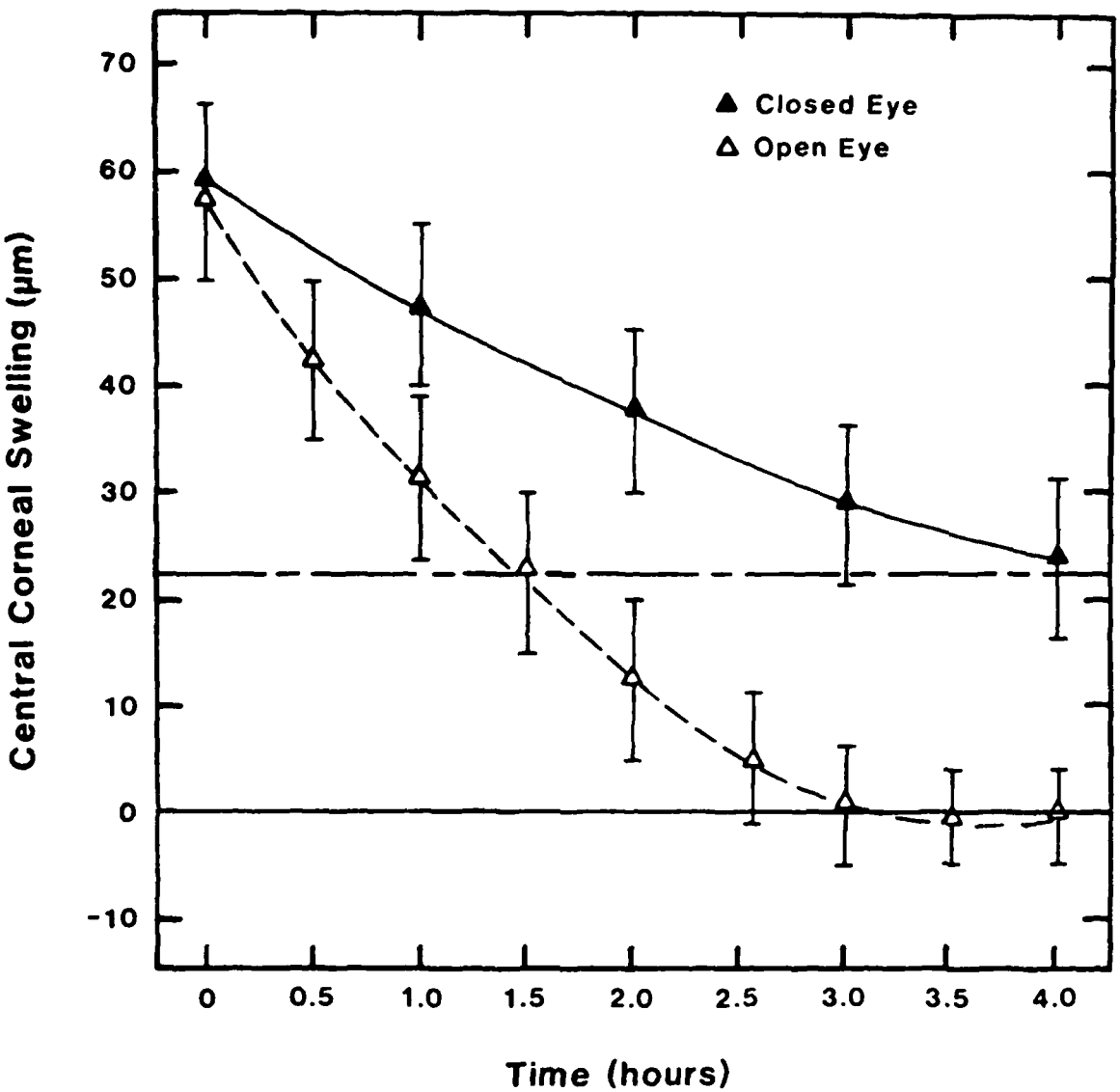

Figure 5.1. Mean decrease in central corneal swelling vs time for 10 older subjects (mean age, 65.7 years) during recovery with the eyes open (open triangles) and closed (filled triangles) following corneal swelling induced with the eyes closed. Error bars equal $\pm$ 1 SD and lines were fitted by polynomial equation.

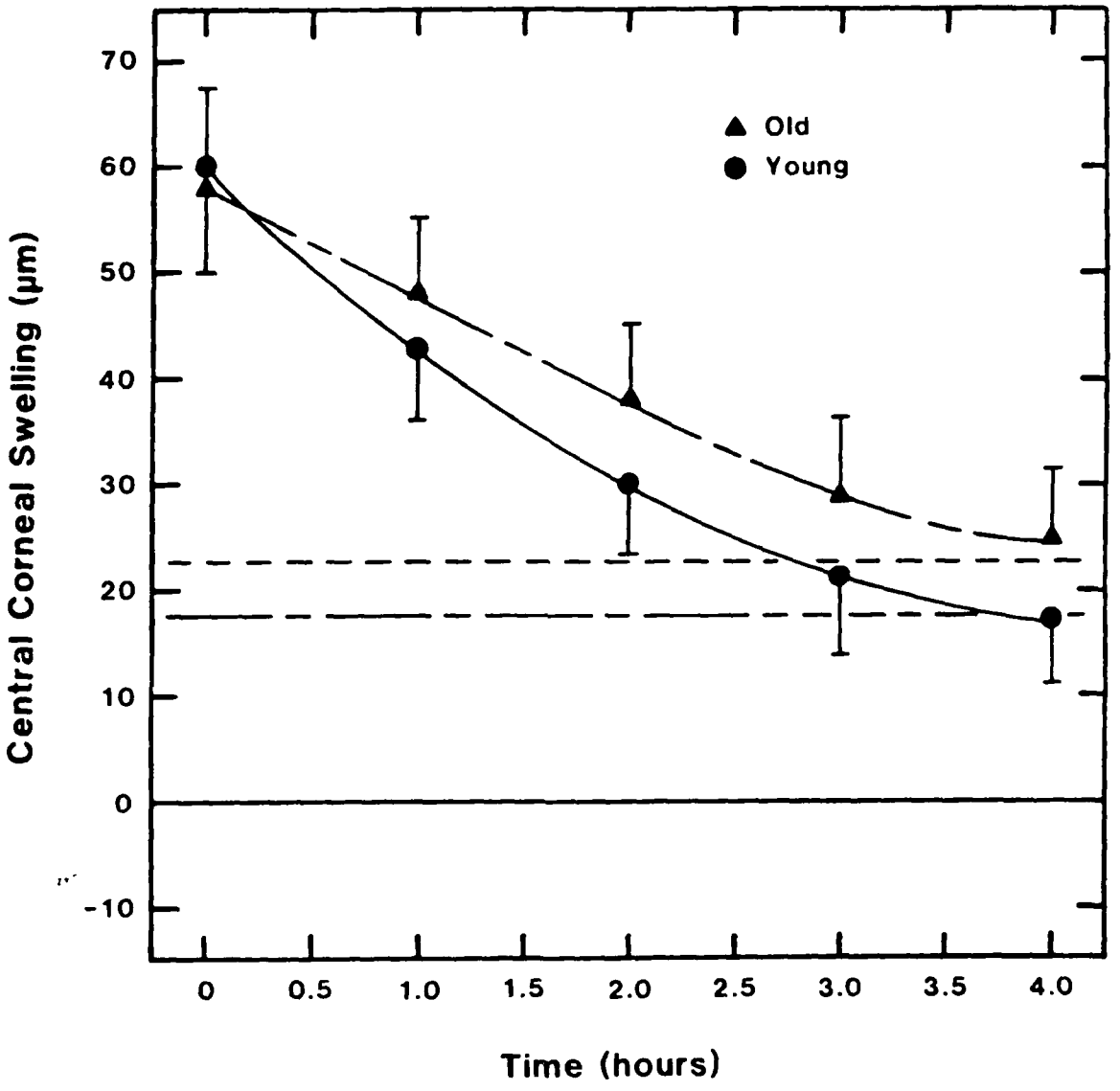

Figure 5.2. Comparison of the mean decrease in central corneal swelling vs time between the old age group (filled triangles) and young age group (filled circles) subjects during recovery with the eyes closed. Error bars equal $\pm$ 1 SD and lines were fitted by polynomial equation.

Using least squares linear analysis, the rate of recovery (μm/hr) over the first 2 hours was computed for each subject, and are listed in Table 5.2 for the older subjects and in Table 5.3 for the younger subjects. The closed eye recovery rate was significantly slower for the older subjects (10.5 $\pm$ 2.9 μm/hr, range 5.5 to 14.0 μm/hr) than for the younger subjects (15.0 $\pm$ 2.2 μm/hr, range 11.5 to 19.0 μm/hr), (Wilcoxon rank-sum, $p < 0.001$).

There is, however, a difference in the mean normal physiologic closed eye swelling of 17.2 μm vs 23.4 μm for the younger and older subjects, respectively. In the younger age group, the closed eye recovery rate was related to the amount of swelling above the physiologic (no lens) swelling that occurs with eye closure [O'Neal and Polse, 1985]. To give the same amount of initial swelling above the normal closed eye level (35 μm) the mean starting edema for the younger age group was adjusted to 52 μm.

Using these adjusted data, Figure 5.3 was constructed to compare the mean recovery during eye closure for both study groups. For the younger group, recovery took 3.5 hours to reach the normal closed eye level; while for the older group, recovery was not complete at the end of the 4 hour test period. Figure 5.3 also shows that the younger group had 1.5 μm of swelling remaining after 3 hours, while the older group did not reach this level of recovery until 4 hours.

Table 5.2. Rate of Recovery (μm/hr) during the initial 2 hr of closed eye recovery and 1 hr of open eye recovery for each of the older age group (mean age, 65.7 yr) subjects.

Subject	Closed Eye Recovery	Open Eye Recovery
1	10.0	36.0
2	13.0	40.0
4	9.5	24.0
5	12.0	19.0
6	9.5	25.0
7	13.0	31.0
8	5.5	21.0
9	12.5	26.0
10	5.5	17.0
3	14.0	25.0
Mean	10.5	26.5
SD	2.9	6.9

Table 5.3. Rate of Recovery (μm/hr) during the initial 2 hr of closed eye recovery and 1 hr of open eye recovery for each of the younger age group (mean age, 26.7 yr) subjects.

Subject	Closed Eye Recovery	Open Eye Recovery
1	14.0	31.0
8	14.0	32.0
3	19.0	37.0
4	15.5	39.0
5	17.5	31.0
6	14.0	41.0
7	12.5	36.0
9	11.5	36.0
10	16.0	35.0
2	15.5	38.0
Mean	15.0	35.6
SD	2.2	3.4

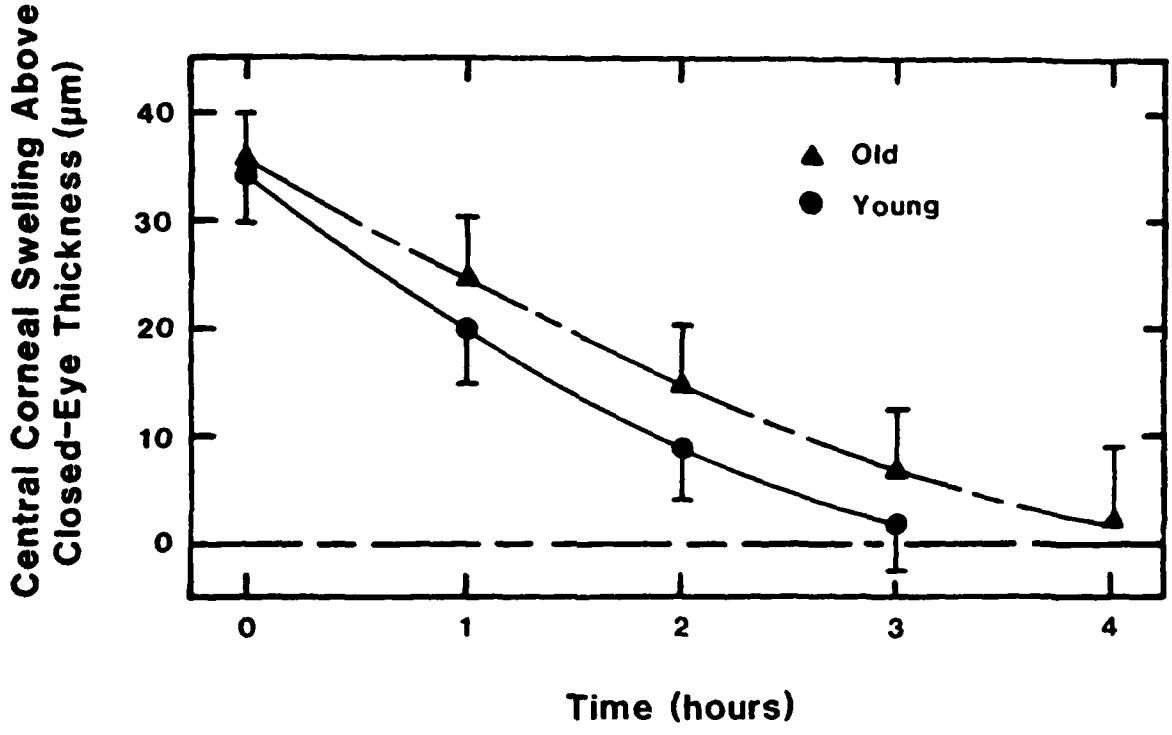

Figure 5.3. Comparison of the mean decrease in central corneal swelling vs time between the older (triangles) and younger (circles) subjects during recovery with the eyes closed from the same amount of edema, 35 μm, above the closed eye corneal thickness.

From the adjusted edema level, the mean rate of recovery over the first 2 hours for the younger subjects was 13.5 μm/hr (range 10.0 to 17.5 μm/hr), which is also significantly faster compared to the older age group rates above (Wilcoxon rank-sum, $p < 0.022$).

Comparison of open eye recovery for both groups of subjects is shown in Figure 5.4. From approximately 60 μm of swelling, recovery to baseline thickness required 2.5 hours and 3.0 hours for the younger and older age groups, respectively. The main difference in recovery profiles between the two groups occurred over the first hour. Using least squares linear analysis, the rate of recovery (μm/hr) over the first hour was computed for each subject, and are listed in Table 5.2 for the older subjects and in Table 5.3 for the younger subjects. The open eye recovery rate was significantly faster for the younger subjects (35.6 ± 3.4 μm/hr, range 31.0 to 41.0 μm/hr) compared to the older subjects (26.5 ± 6.9 μm/hr, range 17.0 to 40.0 μm/hr), (Wilcoxon rank-sum, $p < 0.003$).

For these older subjects, there was considerable intersubject variability in the time course of recovery during both recovery conditions. For example, Figure 5.5 shows the open and closed eye recovery curves for subjects 7 and 10. Subject 10 showed slower recoveries and Subject 7 faster recoveries than the group mean responses. Comparison of the standard deviations in Tables 5.2 and 5.3 indicates a higher variability in recovery rates for the older subjects,

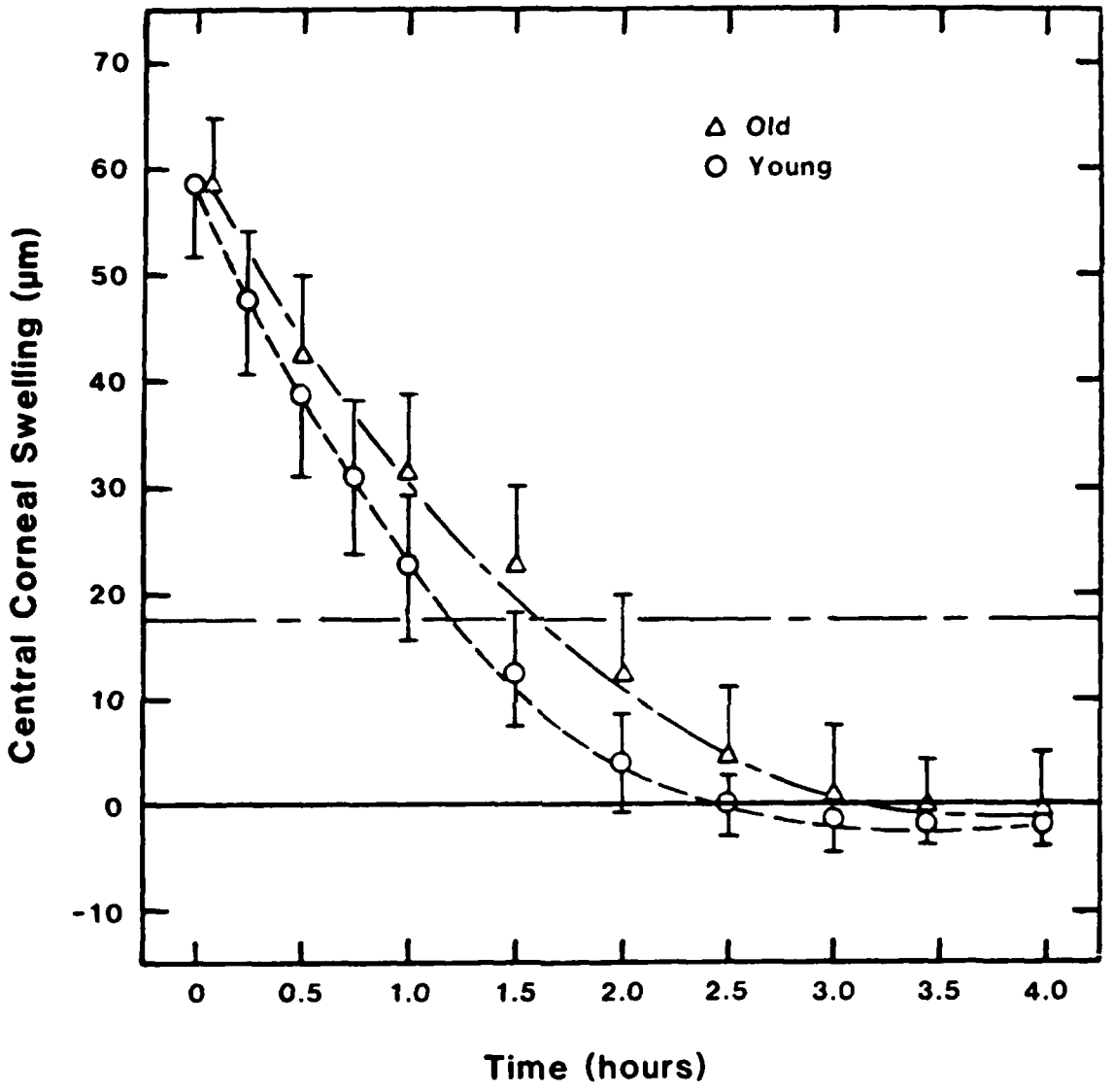

Figure 5.4. Comparison of the mean decrease in central corneal swelling vs time between the older (triangles) and younger (circles) subjects during recovery with the eyes open. Error bars equal ± 1 SD and lines were fitted by polynomial equation.

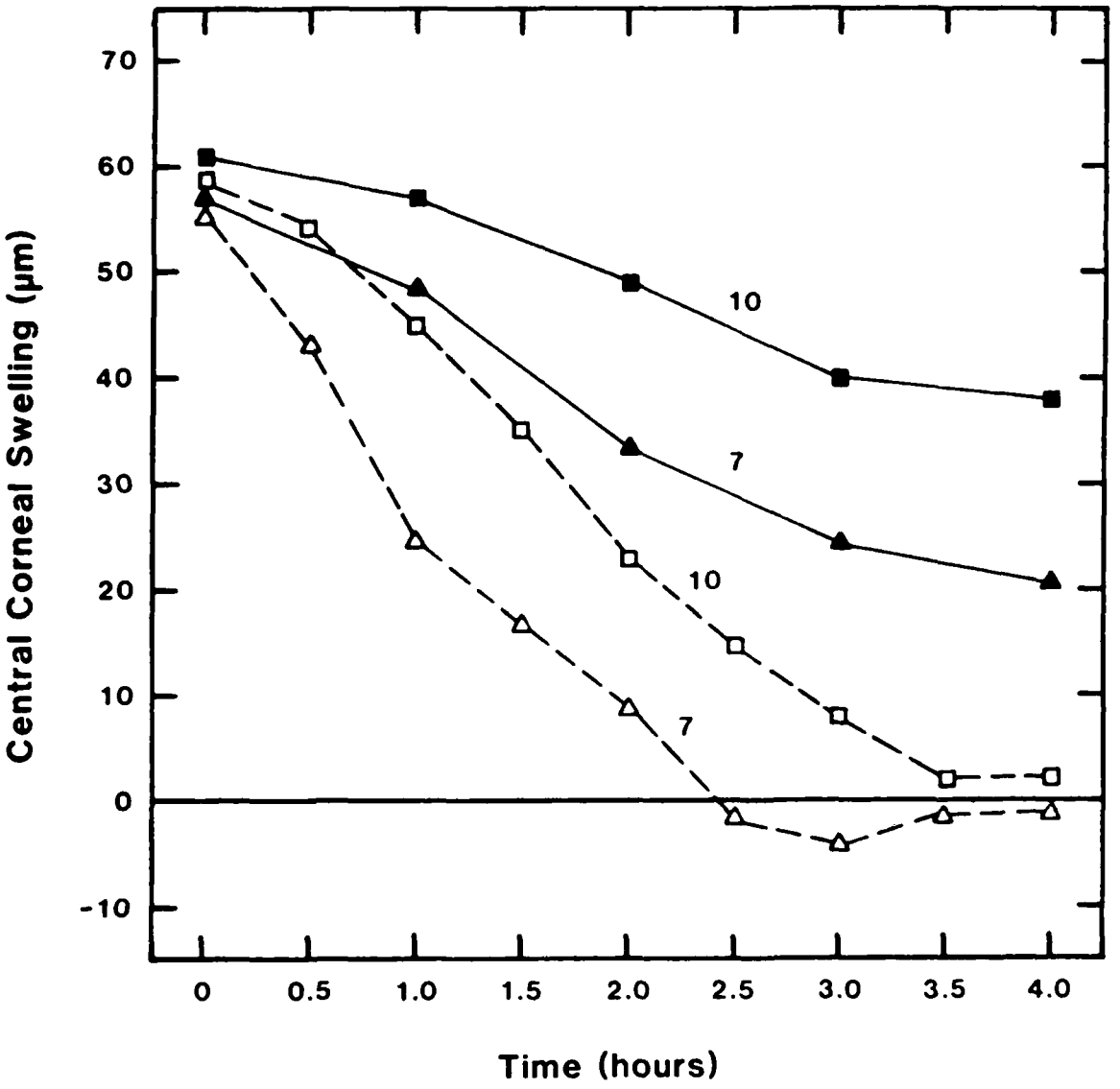

Figure 5.5. Individual variation in decrease in central corneal swelling vs time for 2 older subjects,(7, triangles; 10, squares) during recovery with the eyes closed (filled figures) and open (open figures).

suggesting that a larger population of elderly subjects may be needed to more accurately establish the normative recovery data for this age group.

Endothelial cell photomicrograph tracings showed that the younger subjects tended to have fairly uniform cell size (Figure 5.6), while the older subjects had considerable variation in cell size (Figure 5.7). Analysis of these photomicrographs for mean values and ranges of cell area and coefficient of variation in cell area are compared between the two groups in Table 5.4. Mean cell area in the older subjects was 420 $\pm$ 106 μm^2 (range 313 to 747 μm^2) and was 341 $\pm$ 40 μm^2 (range 300 to 432 μm^2) in the younger subjects. The difference between the two groups was significant (Wilcoxon rank-sum, $p < 0.01$). This corresponds to an endothelial cell density in the older subjects of from 1,339 to 3,195 cells/mm^2 (mean = 2,381) and from 2,315 to 3,333 cells/mm^2 (mean = 2,933) in the younger subjects. The coefficient of variation in cell area ranged from 30.9 to 44.4 (mean = 36.0) in the older subjects, while in the younger subjects the range was from 26.3 to 35.1 (mean = 29.9). The difference between the two groups was significant (Wilcoxon rank-sum, $p < 0.001$).

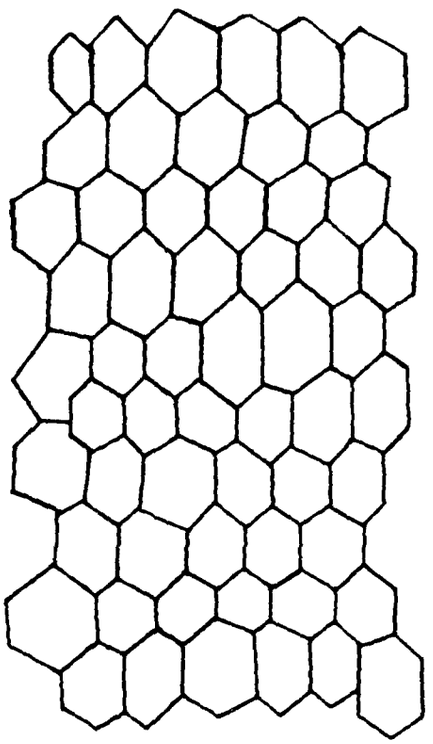

Figure 5.6. Endothelial photomicrograph tracing of 25 year old man, demonstraing uniformity of cell size. Cell density, 3,096 cells/mm^2; Mean cell size, 323 μm^2; Coefficient of variation, 26.3.

AD-A169 334 IN VIVO ASSESSMENT OF MECHANISMS CONTROLLING CORNEAL HYDRATION(U) HARRY G ARMSTRONG AEROSPACE MEDICAL RESEARCH LAB WRIGHT-PATTERSON AFB OH M R O'NEAL JAN 86 2/2

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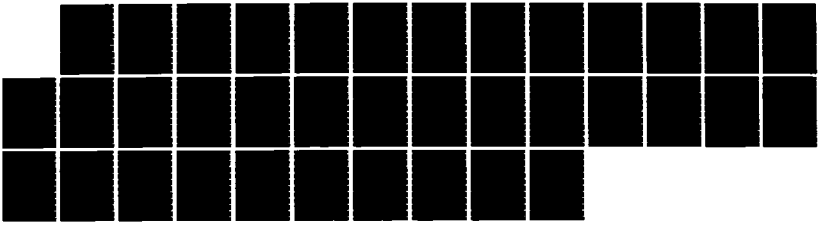

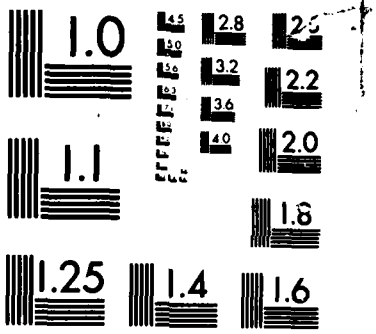
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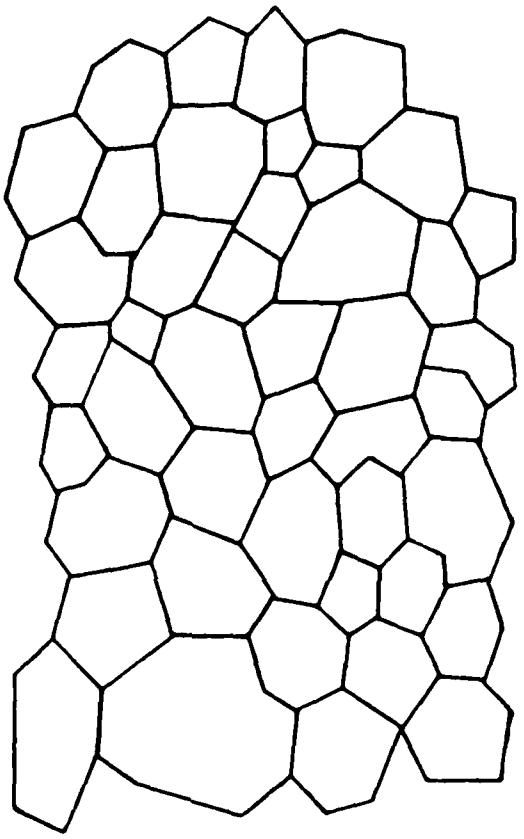

Figure 5.7. Endothelial photomicrograph tracing of 65 year old man, demonstrating marked variation in cell size. Cell density, 2,000 cells/mm^2, Mean cell size, 500 μm^2, Coefficient of variation, 43.2.

Table 5.4. Endothelial cell morphology of the older age group (mean age, 65.7 years) and younger age group (mean age, 26.7 years) subjects.

Characteristic	Older Age Group	Younger Age Group
Mean Cell Area (um^2) (range)	420 (313-747)	341 (300-432)
	($p < 0.01$)	
Mean Cell Density ($cells/mm^2$) (range)	2,381 (1,339-1,195)	2,933 (2,315-3,333)
	($p < 0.01$)	
Mean Coefficient of Variation (range)	36.0 (30.9-44.4)	29.9 (26.3-35.1)
	($p < 0.001$)	

The correlation between endothelial cell morphology and recovery rate with respect to age for the two age groups both separately and combined is shown in Table 5.5. For the combined groups, the rate of recovery was better correlated (negatively) to the coefficient of variation in cell area, $r = -0.62$ ($p < 0.01$) and -0.69 ($p < 0.01$), than was the mean cell area, $r = -0.27$ and -0.55 ($p < 0.05$), for both closed and open eye recovery, respectively. Within the younger age group, however, the coefficient of variation showed little correlation to the rate of recovery with the eyes closed ($r = -0.16$) or open ($r = -0.22$). None of the correlation coefficients for each subject group alone was significant.

The correlation between recovery rate and an individual endothelial morphological characteristic was studied by forming two groups of subjects, without regard to age, in which one characteristic remained within selected limits while the other varied over a wide range, Table 5.6. The groups were: (1) similar coefficient of variation, 32 ± 3.5, mean cell area 301-525 um, (n=11, 4 older, 7 younger); and (2) similar mean cell area, 330 ± 25 um, coefficient of variation 26.3-38.6, (n=11, 5 older,6 younger). Recovery rate was not significantly correlated with mean cell area, $r = -0.27$ and $r = -0.50$, for both closed and open eye recovery, respectively, nor with the coefficient of variation ($r = -0.48$) for open eye recovery. The only significant correlation was between the coefficient of variation and closed eye recovery rate, $r = -0.66$ ($p < 0.05$). (See Appendixes 13-15 for morphology data).

Table 5.5. Correlation (r) between endothelial cell morphology and the rate of recovery over the first 2 hours during eye closure and 1 hour with the eyes open for both age groups individually and combined.

	Closed Eye Recovery vs (r =)		Open Eye Recovery vs (r =)	
	Cell Area	Cof. Var.	Cell Area	Cof. Var.
Younger Age Group	+0.47	-0.16	-0.60	-0.22
Older Age Group	-0.12	-0.32	-0.32	-0.56
Combined Age Groups	-0.27	-0.62 ($p < 0.01$)	-0.55 ($p < 0.05$)	-0.69 ($p < 0.01$)

Table 5.6. Correlation (r) between isolated endothelial cell morphology (one characteristic held within limits while other varied) and the rate of recovery over the first 2 hr during eye closure and 1 hr with the eyes open, without regard to age.

Morphological Characteristic	Closed Eye	Open Eye
Mean Cell Area (301 - 525 um^2) (Coef Var = 32 $\pm$ 3.5)	-0.27	-0.50
Coefficient of Variation (26.3 - 38.6) (Area = 330 $\pm$ 25 um^2)	-0.66 ($p < 0.05$)	-0.48

5.5 Discussion

The rate of corneal recovery following induced edema was slower in the older age group compared to the younger subjects. These recovery rate differences are apparently related to a reduced endothelial function, which could result from a decrease in the active pump or barrier mechanisms. Analysis of these recovery data suggests that the most likely cause of this reduced function is a decrease in the endothelial pump rate.

This conclusion is based on the following analysis. First, in the previous study [O'Neal and Polse, 1985] it was found that after lens removal the rate of recovery with the eyes closed was in close correspondence to the calculated recovery rate due the endothelial pump. However, for these older subjects, the measured rate of recovery was slower than that calculated for the pump. For instance, at the initial 60 μm of swelling the calculated recovery rate is 19 μm/hr; however, the measured rate of recovery was only 12 μm/hr. After 1 hour of recovery, the difference in rates was still 14 vs 10 μm/hr for the calculated and measured recovery rates, respectively. This finding suggests that on the whole the endothelial pump of the older subjects is moving fluid out of the cornea at a reduced rate in comparison to the younger subjects.

Second, the rate of recovery with the eyes open was also slower in the older group compared to the younger subjects,

but only until their normal physiologic closed eye swelling level was reached. From this point the open eye recovery to baseline thickness was the same for both groups. Both the endothelial pump and evaporation contribute to the initial portion of recovery [O''Neal and Polse, 1985], while the later phase of thinning is due entirely to evaporation [Mishima & Maurice, 1961]. Had there been a decreased barrier function, we might expect this later portion of recovery to also be slower in the older subjects. This was not the case, suggesting that the endothelial barrier function remains largely intact with normal aging; which is consistent with studies on the endothelial permeability to fluorescein [Bourne et al, 1984; Sawa et al, 1983].

The reduction in endothelial pump rate can be estimated using the closed eye recovery data. Assuming the endothelial hydraulic conductivity remains normal with aging and given that the mean baseline corneal thickness (509 μm) was the same for the two age groups, then the fluid leak into the cornea due to the stromal swelling pressure would also be equal. Since the leak is the same for both groups, any decrease in recovery rate would be due to a reduced endothelial pump rate. At the initial 60 μm of swelling, the recovery rate for the older subjects is 7 μm/hr less than for the younger group. This corresponds to a decrease in pump rate of 0.7 $\mu l/cm^2$ x hr. The endothelial pump rate reported for the rabbit is 6.7 $\mu l/cm^2$ x hr [Baum et al, 1984]; and taking this rate as normal for the young subjects [O'Neal and Polse,

1985], gives an endothelial pump rate of approximately 6.0 $\mu l/cm^2$ x hr, or a 10% decrease, for the older subject group.

The difference in mean cell size between the two groups can also be used to estimate the reduction in pump rate [Bourne and Brubaker, 1983]. When cell size increases by a factor of X, the total cell perimeter and intercellular space of the corneal endothelium decreases by the square root of X divided by X. The mean cell size for the older subjects (420 μm) was 1.23 times that of the younger age group (341 μm), giving a calculated total intercellular space for the older subjects that is 90% of that in the younger subjects. Since the endothelial transport mechanism appears to be based on the Na,K-ATPase pump [Fischbarg and Lim, 1984] located in the lateral cell membrane [Kaye and Tice, 1966], this 10% reduction in intercellular space, and presumeably pump sites, would be consistent with the apparent decrease found in the pump rate.

The results of the endothelial photomicrograph analysis were variable. The mean cell area was found to be both positively and negatively correlated with recovery rate for the closed and open eye conditions, respectively, in the younger subjects; and had only slight to no correlation within the older subjects. The coefficient of variation in cell size was not correlated with either open or closed eye recovery rates for the younger subjects; however, it was the best indicator of the rate for the older subjects. When the groups

were combined, the coefficient of variation in cell size showed good correlation to recovery rate for both the open and closed eye conditions. When age was disregarded and recovery correlated over a range of values for one morphological characteristic while the other remained within selected limits, mean cell area was again not significantly correlated with recovery rate; while the only significant correlation was between the coefficient of variation and the rate of recovery during eye closure (ie. when recovery is due only to the pump mechanism). These findings suggest that endothelial pump function is affected more by an increase in the relative variability in cell size (ie. polymegathism) than by the average cell size, although an interference with function by the latter characteristic cannot be excluded.

The finding that mean cell area (and cell density) is not correlated with pump function is in agreement with recent studies on pump site density. The endothelial transport mechanism appears to be based on the Na,K-ATPase pump [Fischbarg and Lim, 1984] located in the lateral cell membrane [Kaye and Tice, 1966]. Geroski and Edelhauser [1984] quantitated the density of these pump sites in the rabbit corneal endothelium and calculated that the lateral cell membrane could accommodate many more pump sites than measured. This would allow a decrease in membrane area without necessarily a loss in pump sites. In addition, Geroski et al [1984] have reported that in human donor tissue the pump site density remained relatively constant with age, although the

number and age distribution of the tissues was not listed. Apparently, the decrease in cell density that occurred in our older subjects had little effect on pump function.

The rate of corneal recovery following induced swelling was slower in the older group of subjects compared to the younger subjects. It is possible that measuring the recovery for each group at different times may have affected the response; however, care was taken to insure the instruments and environmental conditions were identical for both groups. Analysis of these recovery data suggests that endothelial pump function decreases with aging, and that this change may be linked to alterations in endothelial morphology, particularly polymegathism. For instance, in the analysis when each morphological characteristic was isolated, the only significant correlation occurred between closed eye recovery rate and coefficient of variation ($r = -0.66$); however, within this analysis group, the correlation was $r = -0.81$ for the five older subjects, while no correlation ($r = -0.01$) was found for the six younger subjects. Although a small subject number is involved, this suggests that age plays a role in the susceptibility of the pump mechanism to interference from morphological changes. The complete pump mechanism is complex and involves other cell membranes and pump sites than the Na,K-ATPase sites; and the combination of age and polymegathism may be affecting one of the pump components and cause the decrease in overall pump function we found in the older age group.

The difference in recovery rates was apparent after only 1 - 2 hr, indicating only a short monitoring period is needed to evaluate recovery ability. Additional studies on larger populations are needed to establish the normal range of recovery responses and to investigate endothelial pump function in corneas demonstrating endothelial disease.

CONCLUSION

These studies were a progression of investigations conducted with continuous deliberations and discussions with my advisor Dr. Kenneth A. Polse. Upon his suggestion, I began the assessment of the use of contact lenses in a test of corneal function. The journey has led toward understanding and quantifying the mechanisms controlly corneal hydration in the in vivo human cornea, and the development of a test of endothelial function. In this test, corneal hydration recovery is monitored following hydrogel lens induced hypoxia. These studies indicate that this Endothelial Function Test (EFT) is a valid technique for assessing endothelial pump function. The relatively short time period needed to administer the test and good patient comfort during the procedure suggest the method may also have clinical applicability. It is hoped that these studies are just the beginning of research and development of this technique as a method to assess endothelial function.

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APPENDIXES (CHAPTER 3)

INDIVIDUAL DATA

Appendix 1. Central corneal swelling of 8 subjects (1-8) following 3 hours of eye closure while wearing lenses 3-8.

Appendix 2. Central corneal swelling of 6 subjects (9-14) following 3 hours of eye closure while wearing lenses 1, 2, and 9-12.

Appendix 3. Open eye recovery from initial swelling of 70 µm and above during 9 sessions amoung the 14 subjects.

Appendix 4. Open eye recovery from initial swelling of 55-69 µm during 16 sessions amoung the 14 subjects.

Appendix 5. Open eye recovery from initial swelling of 40-54 µm during 11 sessions amoung the 14 subjects.

APPENDIX 1

Central corneal swelling of 8 subjects (1-8) following 3 hours of eye closure while wearing lenses 3-8. (See text for lens specifications.)

Lens No.	Central Corneal Swelling (μm) Subject Number 1	2	3	4	5	6	7	8	$\bar{x}$	SD
3	59	61	82	75	64	62	67	86	69.5	10.2
4	64	61	72	63	75	63	67	75	67.5	5.7
5	72	61	60	60	61	63	67	73	64.6	5.4
6	45	50	53	55	51	49	62	68	54.1	7.5
7	51	44	42	58	47	39	63	67	51.3	10.3
8	51	36	37	37	50	39	53	49	44.0	7.4
None	26	32	17	18	20	15	20	27	21.9	5.8
TLOE	57	43	47	50	40	57	55	63	51.5	7.9

None is no lens, or normal physiologic eye closure

TLOE is Thick Lens Open Eye wear for 3 hours

APPENDIX 2

Central corneal swelling of 6 subjects (9-14) following 3 hours of eye closure while wearing lenses 1, 2, and 9-12. (See text for lens specifications.)

Lens No.	Central Corneal Swelling (μm) Subject Number 9	10	11	12	13	14	$\bar{x}$	SD
1	85	86	75	77	90	-	82.5	6.3
2	86	85	86	71	89	78	82.5	6.8
9	52	50	44	38	35	45	44.0	6.6
10	39	37	34	28	28	31	32.8	4.6
11	-	33	32	26	26	30	29.5	3.9
12	28	27	29	20	19	19	23.5	5.1
None	20	24	20	18	19	18	19.8	2.2
TLOE	56	40	64	58	41	47	51.0	9.8

None is no lens, or normal physiologic eye closure

TLOE is Thick Lens Open Eye wear for 3 hours

* Two sessions discontinued due to subject discomfort

APPENDIX 3

Open eye recovery from initial swelling of 70 µm and above during 9 sessions among the 14 subjects. Corneal swelling was induced with contact lenses worn for 3 hours with the eyes closed and recovery was monitored following lens removal.

Recovery Time (hr)	Central Corneal Swelling (µm) Subject Number (Lens No.) 1 (5)	3 (3)	4 (3)	8 (3)	8 (5)	9 (2)	10 (2)	12 (1)	13 (1)	$\bar{x}$	SD
0	72	82	75	86	73	85	85	77	90	80.6	6.5
0.25	64	62	57	75	57	76	75	65	80	67.9	8.7
0.5	49	44	38	59	43	65	66	56	71	54.6	11.6
0.75	31	37	28	47	35	52	58	47	63	44.2	12.1
1.0	23	30	18	38	29	42	48	38	49	35.0	10.8
1.5	13	9	-1	17	17	27	32	25	34	19.2	11.4
2.0	1	0	-4	8	10	15	18	8	21	8.6	8.5
2.5	-7	-8	-9	4	2	5	8	4	5	0.4	6.5
3.0	-7	-8	-10	0	-2	-5	2	-5	-1	-4.0	4.0
3.5	-5	-6	-8	-5	-4	-9	-1	-3	-5	-5.1	2.4
4.0	-5	-5	-6	-7	-3	-7	-2	-1	-3	-4.3	2.2

APPENDIX 4

Open eye recovery from initial swelling of 55-69 µm during 16 sessions amoung the 14 subjects. Corneal swelling was induced with contact lenses worn for 3 hours with the eyes closed and recovery was monitored following lens removal.

	Central Corneal Swelling (µm)																		
	Subject Number																		
Recovery	(Lens No.)																		
Time	1	2	2	3	4	4	5	5	6	6	7	7	7	7	8	8		$\bar{x}$	SD
(hr)	(3)	(3)	(5)	(5)	(5)	(7)	(3)	(5)	(3)	(5)	(3)	(5)	(6)	(7)	(6)	(7)			
0	59	61	61	60	60	58	64	61	62	63	67	67	62	63	68	67		62.8	7.1
0.25	43	49	51	45	40	45	54	46	48	51	53	55	54	47	55	52		49.3	4.6
0.5	25	35	34	37	22	37	46	38	39	39	42	44	42	43	45	40		38.0	6.7
0.75	23	26	21	20	14	31	36	28	29	32	37	34	32	37	32	35		29.2	6.8
1.0	16	14	11	9	7	22	25	18	24	24	27	27	25	29	27	24		20.8	6.8
1.5	0	4	-3	3	-1	8	11	6	13	14	15	9	11	17	15	15		8.6	6.4
2.0	-3	-4	-7	-3	-9	-4	-1	0	0	0	6	0	6	7	6	5		-0.1	4.9
2.5	-4	-9	-9	-6	-9	-5	-4	-8	-8	-3	-2	-2	-1	-1	-1	-2		-4.9	3.5
3.0	-8	-9	-8	-6	-7	-3	-7	-6	-6	-2	-5	-1	-4	-4	-8	-2		-5.4	2.5
3.5	-7	-9	-7	-3	-4	1	-5	-3	-5	-4	-3	-3	-2	0	-5	-1		-3.8	2.6
4.0	-3	-7	-4	-2	-3	-1	-4	-3	-4	-1	0	-2	-2	-1	-5	-1		-2.7	1.8

APPENDIX 5

Open eye recovery from initial swelling of 40-54 μm during 11 sessions amoung the 14 subjects. Corneal swelling was induced with contact lenses worn for 3 hours with the eyes closed and recovery was monitored following lens removal.

Recovery Time (hr)	Central Corneal Swelling (μm) Subject Number (Lens No.) 1 (6)	1 (7)	2 (6)	2 (7)	3 (6)	3 (7)	4 (6)	5 (6)	5 (7)	6 (6)	6 (7)	$\bar{x}$	SD
0	45	51	50	44	53	42	55	51	47	49	39	47.9	4.9
0.25	35	42	34	32	36	29	43	42	38	37	29	36.1	4.9
0.5	25	22	22	26	22	22	35	29	27	30	24	25.8	4.2
0.75	18	14	17	16	14	19	24	20	19	24	18	18.5	3.4
1.0	10	10	12	6	5	8	12	13	11	17	5	9.9	3.7
1.5	4	1	5	0	0	-2	0	-1	3	9	2	1.9	3.2
2.0	-2	-3	-1	-2	-5	-7	-4	-4	-8	-1	1	-3.3	2.7
2.5	-4	-6	-1	-5	-8	-7	-7	-5	-8	-7	-5	-5.5	2.1
3.0	-6	-4	1	-2	-8	-4	-5	-3	-7	-7	-2	-4.3	2.2
3.5	-2	-2	-2	1	-7	-2	-3	-1	-4	-6	-2	-2.7	2.2
4.0	-2	-3	-2	0	-4	1	-2	0	-2	-5	1	-1.6	2.0

APPENDIXES (CHAPTER 4)

INDIVIDUAL DATA

Appendix 6. Closed eye recovery of 10 younger subjects.

Appendix 7. Open eye recovery of 10 younger subjects.

Appendix 8. Open eye recovery in 100% humidity environment for the 6 additional subjects.

Appendix 9. Closed eye recovery for the 6 additional subjects.

Appendix 10. Open eye recovery in normal (60%) humidity environment for the 6 additional subjects.

APPENDIX 6

Closed eye recovery of 10 younger subjects.

Central Corneal Swelling (μm)												
Recovery Time (hr)	Subject Number											
	1	2	3	4	5	6	7	8	9	10	$\bar{x}$	SD
0	64	64	62	65	56	65	56	57	55	58	60.1	4.2
1.0	48	48	41	53	36	44	45	40	46	38	42.9	4.1
2.0	36	33	24	34	21	37	31	29	32	26	30.3	5.2
3.0	21	19	18	27	19	21	18	22	29	15	20.9	4.3
4.0	17	17	16	19	15	16	18	17	19	14	17.2	3.1

APPENDIX 7

Open eye recovery of 10 younger subjects.

Central Corneal Swelling (μm)												
Recovery Time	Subject Number											
(hr)	1	2	3	4	5	6	7	8	9	10	$\bar{x}$	SD
0	62	56	57	59	60	58	56	63	59	60	59.1	4.4
0.25	48	44	43	54	50	42	46	51	49	48	47.8	3.8
0.5	39	36	34	45	39	34	41	42	39	38	38.7	3.5
0.75	35	25	28	29	34	25	29	34	31	32	30.2	3.6
1.0	31	18	20	20	29	17	20	30	23	25	23.3	5.2
1.5	17	6	13	10	20	7	14	19	10	10	12.6	4.9
2.0	8	-1	1	1	12	4	3	10	4	4	4.6	4.2
2.5	1	-1	-4	-1	2	0	0	2	0	0	-0.1	1.7
3.0	1	1	-3	-3	-1	1	-3	-1	-1	2	-0.6	2.0
3.5	-1	-1	-2	0	-5	0	1	0	-3	-3	-1.4	1.9
4.0	0	-1	0	-3	-3	0	-2	0	-2	1	-1.0	1.5

APPENDIX 8

Open eye recovery in 100% humidity environment for the 6 additional younger subjects.

Recovery Time (hr)	Central Corneal Swelling (μm) Subject Number 1	2	3	4	5	6	$\bar{x}$	SD
0	63	60	60	58	59	61	60.2	1.8
1.0	42	42	45	43	38	46	42.6	2.8
2.0	26	27	27	27	24	30	26.8	2.0
3.0	14	13	17	18	16	20	16.8	2.6
4.0	10	11	14	14	13	16	13.0	2.2

APPENDIX 9

Closed eye recovery for the 6 additional younger subjects.

Recovery Time (hr)	Central Corneal Swelling (μm) Subject Number 1	2	3	4	5	6	$\bar{x}$	SD
0	66	59	59	58	58	61	60.2	3.2
1.0	46	44	47	45	40	48	45.0	2.9
2.0	34	30	31	32	28	32	31.1	2.1
3.0	25	18	18	20	20	22	20.6	2.8
4.0	16	14	14	17	17	19	16.5	2.0

APPENDIX 10

Open eye recovery in normal (60%) humidity environment for the 6 additional younger subjects.

Recovery Time (hr)	Central Corneal Swelling (μm) Subject Number 1	2	3	4	5	6	$\bar{x}$	SD
0	61	63	60	59	60	62	60.8	1.5
0.25	49	51	47	49	48	49	48.8	1.5
0.5	42	39	37	39	38	39	39.0	1.9
0.75	34	32	31	30	32	34	32.2	1.6
1.0	29	24	27	22	25	26	25.5	2.4
1.5	19	14	14	10	10	12	13.2	3.4
2.0	10	0	4	2	4	2	3.7	3.7
2.5	2	-3	-1	-1	0	-2	-0.8	1.9
3.0	-1	-2	0	-2	2	-5	-1.3	2.3
3.5	0	-4	0	-2	-3	-3	-2.0	1.8
4.0	0	-1	-1	-1	1	-2	-0.8	1.1

APPENDIXES (CHAPTER 5)

INDIVIDUAL DATA

Appendix 11. Closed eye recovery of 10 older subjects.

Appendix 12. Open eye recovery of 10 older subjects.

Appendix 13. Rates of recovery over the first 2 hours of closed eye recovery, endothelial mean cell area, and coefficient of variation in cell area of the 10 older subjects.

Appendix 14. Rates of recovery over the first 1 hour of open eye recovery, endothelial mean cell area, and coefficient of variation in cell area of the 10 older subjects.

Appendix 15. Rates of recovery over the first 2 hours of closed eye recovery, 1 hour of open eye recovery, endothelial mean cell area, and coefficient of variation in cell area for the right eye of the 10 younger subjects.

APPENDIX 11

Closed eye recovery of 10 older subjects.

Recovery Time (hr)	Central Corneal Swelling (μm) Subject Number 1	2	3	4	5	6	7	8	9	10	$\bar{x}$	SD
0	56	60	65	54	56	61	57	57	60	60	58.6	4.8
1.0	41	45	50	44	40	51	49	52	47	57	47.6	5.1
2.0	36	34	37	35	32	42	31	46	35	49	37.9	5.5
3.0	30	23	24	29	27	32	25	40	21	40	29.3	6.1
4.0	25	13	18	19	26	29	21	36	19	38	24.4	7.8

APPENDIX 12

Open eye recovery of 10 older subjects.

Recovery Time (hr)	Central Corneal Swelling (μm) Subject Number 1	2	3	4	5	6	7	8	9	10	$\bar{x}$	SD
0	58	58	62	54	54	60	56	57	63	62	58.6	4.1
0.5	40	25	44	39	45	40	42	44	49	56	42.4	7.6
1.0	22	18	37	30	35	35	25	36	37	45	32.0	7.9
1.5	13	11	26	24	26	28	17	23	25	35	22.8	6.9
2.0	6	1	14	11	15	22	10	13	15	22	12.9	6.2
2.5	0	2	8	4	6	15	-3	5	8	14	5.7	5.6
3.0	-8	0	0	0	0	8	-5	0	3	8	0.6	4.8
3.5	-1	-3	-1	0	-5	5	-1	1	-2	0	-0.7	2.6
4.0	-4	-1	-6	2	0	1	-1	0	-2	3	-0.8	2.7

APPENDIX 13

Rates of recovery over the first 2 hours of closed eye recovery, endothelial mean cell area, and coefficient of variation in cell area of the 10 older subjects.

Subject-Eye	Recovery Rate (μm/hr)	Mean Cell Area (μm^2)	Coefficient of Variation
1 - L	10.0	331	38.6
2 - R	13.0	343	30.9
3 - R	14.0	354	31.9
4 - L	9.5	525	34.8
5 - R	12.0	500	43.2
6 - R	9.5	417	42.4
7 - L	13.0	394	38.6
8 - L	5.5	313	38.3
9 - L	12.5	343	35.0
10 - R	5.5	456	37.3

APPENDIX 14

Rates of recovery over the first 1 hour of open eye recovery, endothelial mean cell area, and coefficient of variation in cell area of the 10 older subjects.

Subject-Eye	Recovery Rate (μm/hr)	Mean Cell Area (μm^2)	Coefficient of Variation
1 - R	36.0	335	36.4
2 - L	40.0	356	32.3
3 - L	25.0	394	34.3
4 - R	24.0	579	34.7
5 - L	19.0	435	44.4
6 - L	25.0	747	32.0
7 - R	31.0	415	32.3
8 - R	21.0	362	40.6
9 - R	26.0	329	33.7
10 - L	17.0	394	34.3

APPENDIX 15

Rates of recovery over the first 2 hours of closed eye recovery, 1 hour of open eye recovery, endothelial mean cell area, and coefficient of variation in cell area for the right eye of the 10 younger subjects. Left eye served as control.

Subject	Recovery Rate (μm/hr) Closed Eye	Open Eye	Mean Cell Area (μm^2)	Coefficient of Variation
1	14.0	31.0	323	32.2
2	15.5	38.0	330	27.0
3	19.0	37.0	324	31.8
4	15.5	39.0	323	26.3
5	17.5	31.0	432	28.7
6	14.0	41.0	301	33.3
7	12.5	36.0	342	28.7
8	14.0	32.0	350	35.1
9	11.5	36.0	300	29.8
10	16.0	35.0	382	26.5

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